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A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA SORGHI [1]

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INTRODUCTION

The existence of heterothallism in rusts (*22*) [3] and the function of the pycnia (*23*) were announced by Craigie in 1927. Stimulated by this discovery, a number of workers entered the field. There is now evidence that the following rusts are heterothallic: *Puccinia graminis* Pers. (*2, 7, 22, 23, 24, 35, 37, 40, 41, 45*), *P. triticina* Eriks. (*3*), *P. helianthi* Schw. (*15, 22, 23, 24, 25*), *P. coronata* Cda. (*4, 24*), *P. pringsheimiana* Kleb. (*24*), *Gymnosporangium* sp. (*24*), *Melampsora lini* (Pers.) Lév. (*5, 8*), *Puccinia sorghi* Schw. (*6, 26, 44*), *Cronartium ribicola* Fisch. (*42*), *Uromyces appendiculatus* (Pers.) Fries (*9, 10*), *U. vignae* Barclay (*9, 10*).

Cytological studies of some of these rusts by Andrus (*9, 10*), Hanna (*35*), Rice (*44*), Pierson (*43*), and Allen (*2, 3, 4, 5, 6, 7, 8*) have contributed to an understanding of the sex mechanisms in rusts, but much still remains to be learned.

Corn rust, *Puccinia sorghi*, has proved favorable material. It is a full-cycle, heteroecious rust, alternating between corn and *Oxalis*. Mains, Trost, and Smith (*39*) made extensive tests to determine types and degrees of resistance and susceptibility of corn varieties to *P. sorghi*. That it is heterothallic has been proved by Cummins (*26*), who found that nearly all isolated haploid infections remained sterile, that when the combined exudate of many infections was brought to an infection it developed aecia, but that if this exudate was first filtered to remove the spermatia no aecia resulted. Rice (*44*), who studied cytologically the aecial generation of *P. sorghi*, notes (*44, p. 40*) that "There are, here and there on the leaf surfaces, cases where a spermatium seems to have germinated." She finds stomatal hyphae, especially in the stomata adjoining spermogonia and aecia, and an occasional binucleate cell in substomatal hyphae connected with

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[3] Reference is made by number (italic) to Literature Cited, p. 1066.

stomatal hyphae; and she records (*44, p. 41*) the presence of "many fusions and occasional migrating nuclei" in young fertile aecia. It was in the hope of confirming and adding to this information that the present study of corn rust has been undertaken.

MATERIALS AND METHODS

Plants of *Oxalis corniculata* L. var. *atropurpurea* Planch. were grown in 6-inch pots in the greenhouse and out of doors on a balcony. Leaves of corn, *Zea mays* L., bearing telia of *Puccinia sorghi*, were weathered during the winter by placing them on the surface of damp soil out of doors in a partly shaded spot. In inoculating, a glass tube 4 inches in diameter and lined with wet paper toweling was set upright over the oxalis plant with the lower edge of the tube pressed into the soil. A 4-inch Petri dish was filled with mud, the corn leaves were pressed into the mud with the telia exposed, and the Petri dish inverted and placed on the upper end of the tube as a lid. Wet paper toweling was folded down over the Petri dish and top of the tube and held in place by a rubber band. This brought the teliospores directly over the oxalis plant in a small damp chamber. The whole was then placed in a shaded spot and kept there for 48 hours, after which the tube was removed and the plants placed in cages covered with tarlatan to exclude insects. Part of the infections were used for experiments in the greenhouse and the rest were fixed at different ages for cytological study. The fixing fluids used were Flemming's medium solution, full strength and two-thirds strength, and Flemming's weak solution. After 1½ or 2 days in the fixing fluid, the material was washed, dehydrated, and embedded in 50° paraffin. The stains used were principally (1) iron haematoxylin counterstained with Congo red, and (2) a combination of saffranine and methylene blue.

INVESTIGATIONS

THE HAPLOID INFECTION

When the sporidium of *Puccinia sorghi* falls upon an oxalis leaf, under conditions suitable for further development, it pierces the outer epidermal wall, and the sporidial contents flow through into the epidermal cell and develop there into a short hypha. Plate 1, *A*, shows an epidermal cell containing a primary hypha fixed 3 days after inoculation. *A*, *a*, is the remnant of the sporidial wall, collapsed and displaced. From the point of entrance at *b* the hypha grew down into the cell, then recurved to *c*. Intracellular growth is short-lived, and the first-formed cells of this primary hypha are already decadent. Branches from the primary hyphae at *d*, *e*, and *f* have grown down to the inner wall of the epidermis, ready to push through into the subepidermal region and establish there the intercellular mycelium.

EXPLANATORY LEGEND FOR PLATE 1

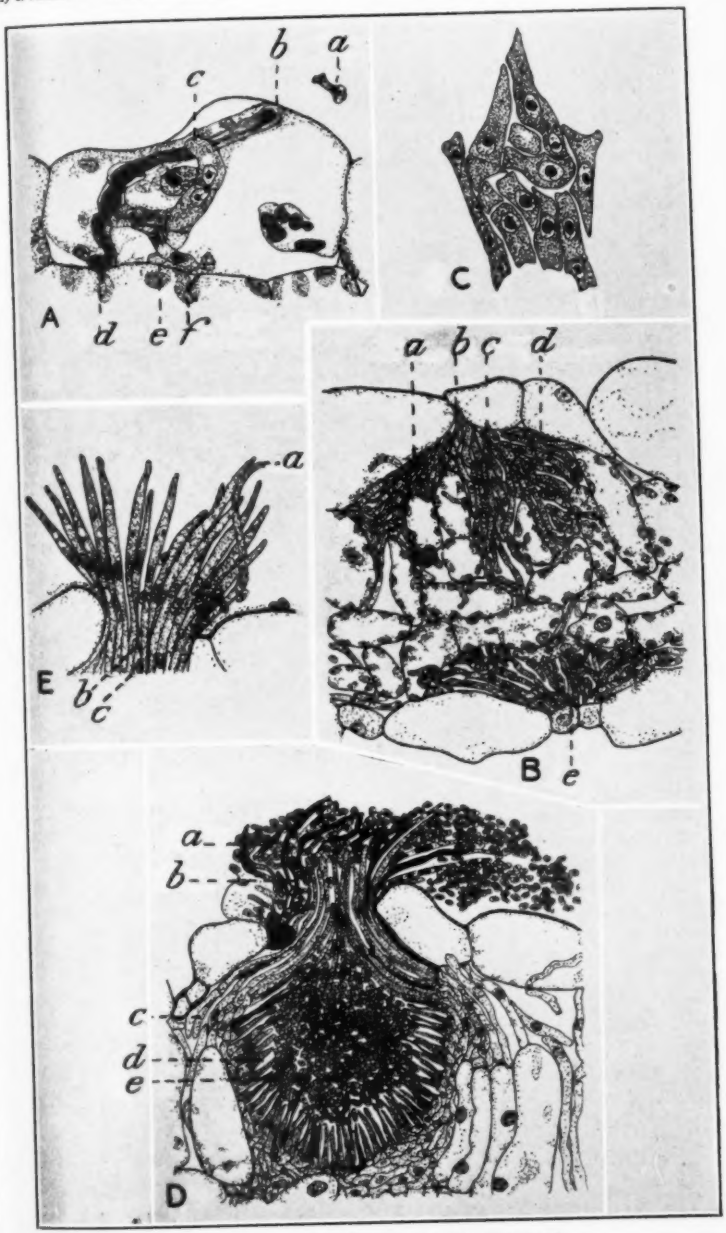
A.—Remnant of sporidium, *a*, from which was developed primary hypha, *b*, *c*, with its branches, *d*, *e*, *f*, in an epidermal cell. ×1,020.

B.—Two stages of development of the spermogonium, *e* and *a*, *b*, *c*, *d*. ×460.

C.—Detail of spermogonium. ×1,400.

D.—Mature spermogonium with decadent paraphyses, *a*, young secondary paraphyses, *b*, wall of spermogonium, *c*, spermatiophores, *d*, and spermatia, *e*. ×460.

E.—Young paraphyses with an irregular paraphysis, *a*, and spermatia, *b*, c. ×460.



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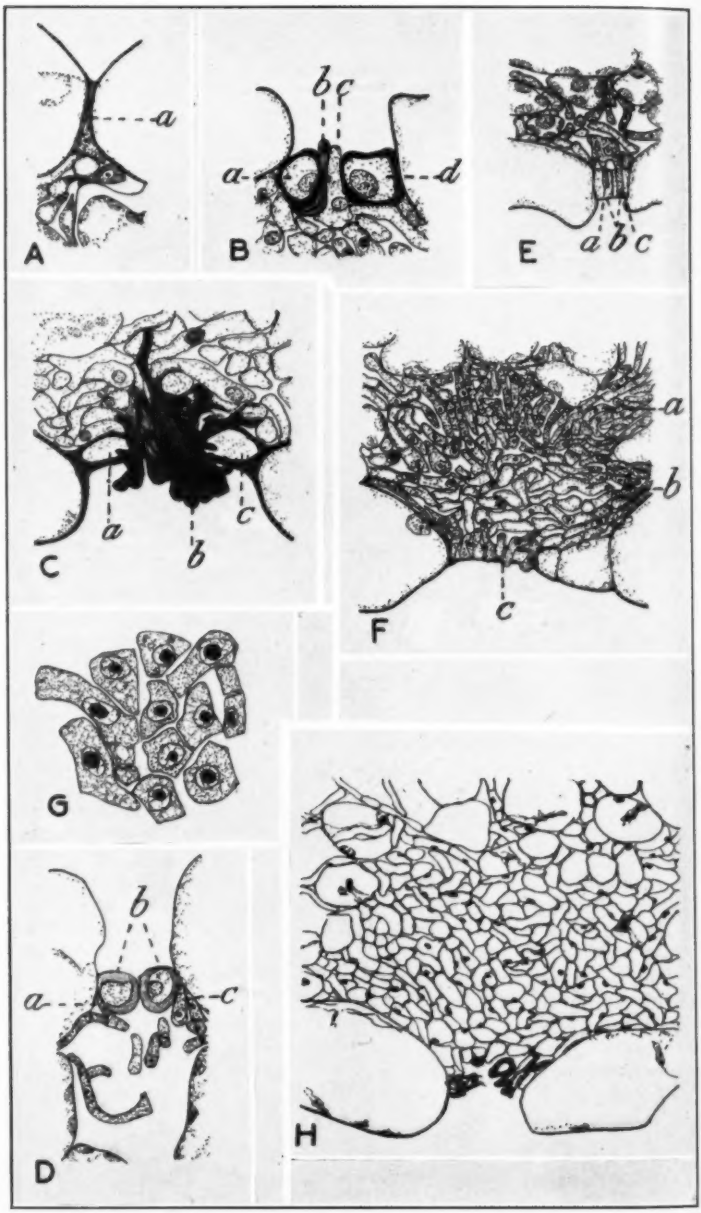

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This mycelium grows rapidly, spreading through the air spaces of the leaf. The first visible indication of infections on living leaves are minute whitish flecks appearing on the sixth or seventh day after inoculation. These become rapidly larger and more discolored.

Both the primary hypha and the vegetative mycelium to which it gives rise consist of uninucleate cells. A count of 100 cells in isolated infections 11 days old shows that 94 are uninucleate and 6 binucleate. When a cell is about to divide, its nucleus divides first, so that the cell is momentarily binucleate. The 6 percent of binucleate cells is no more than would be expected in growing mycelium.

Six or seven days after inoculation, when the vegetative mycelium of the rust is well established in the oxalis leaf, reproductive activity begins. Hyphae from the intercellular spaces of the mesophyll tissue grow outward to the epidermis and form there definite little subepidermal mats of hyphae, the spermogonial anlagen. The earliest anlagen form at the upper epidermis (pl. 1, *B*, *b*), but these are soon followed by others at the lower epidermis (pl. 1, *B*, *e*). These, like the haploid mycelium producing them, consist of uninucleate cells. In plate 1, *C*, is drawn a detail at high magnification. Each cell has dense cytoplasm and a single large spherical or ellipsoidal nucleus. The nucleus contains a very delicate chromatin net and a single large central spherical nucleole.

The development of the spermogonium differs in no important particular from that of the other cereal rusts studied. Hyphae from all parts of the mat grow outward, lifting the epidermis and slanting toward a common center as they grow. The point of convergence (pl. 1, *B*, *b*) marks the location of the future ostiole. The hyphae nearest the epidermis and running parallel to it (pl. 1, *B*, *a*, *d*) will meet at *b*, turn outward, break through the epidermis and form an external brush of hyphae, the paraphyses (pl. 1, *E*). The interior part (pl. 1, *B*, *c*) develops rapidly into the body of the spermogonium, which at maturity consists of a hollow hemispherical wall (pl. 1, *D*, *c*) from which hundreds of short spermatiophores, *d*, project into the central cavity. These give rise to spermatia which fill the cavity, *e*, and then ooze out through the ostiole in a viscous liquid forming the external drop of spermogonial exudate, *a*. So far as noted, the spermogonium throughout its development consists of uninucleate cells, although as a rare exception a spermatium may be binucleate.

The first spermogonia open on the eighth day after inoculation. The period of activity of the individual spermogonium is rather short, although in the absence of fertilization it may continue for some time. The slender tapering paraphyses (pl. 1, *E*) are short-lived, but as they deteriorate and die (pl. 1, *D*, *a*) they are replaced by a fresh growth from the interior (pl. 1, *D*, *b*).

Spermogonia continue to form as the area of infection expands. One hundred spermogonia have been counted in six infections 11

EXPLANATORY LEGEND FOR PLATE 2

A.—Hypha, *a*, between epidermal cells. ×1,020.
B.—Hyphae, *b*, *c*, in stoma between guard cells, *a*, *d*. ×1,020.
C.—Dead stomatal hyphae, *b*, between guard cells, *a*, *c*. ×1,020.
D.—Hyphae, *a*, *c*, pushing out at side of stoma, *b*. ×1,020.
E.—Beginning of haploid aecium with hyphae, *a*, *c*, at end of stoma, *b*. ×460.
F.—Haploid aecium with sporogenous area, *a*, space-making area, *b*, and stomatal hyphae, *c*. ×460.
G.—Detail from sporogenous area of haploid aecium. ×1,400.
H.—Decadent haploid aecium. ×460.

days of age. Fifty-seven of these spermogonia opened upon the upper surface of the leaf and 43 upon the lower. In the absence of fertilization, spermogonia continue to form for a longer period, and those formed later are predominantly on the lower side of the leaf. One exceptionally large 30-day sterile infection bore 318 spermogonia, of which 125, or 39 percent, opened upon the upper leaf surface, and 193, or 61 percent, on the lower surface. Some of these spermogonia were still active.

Spermogonial exudate is not abundant in *Puccinia sorghi*, under the conditions of these experiments, at least. Ordinarily, the secretion of each spermogonium is maintained as a minute independent drop, so that to the naked eye the surface of an infection appears dotted with minute glistening specks (fig. 1). Rarely the secretions of the

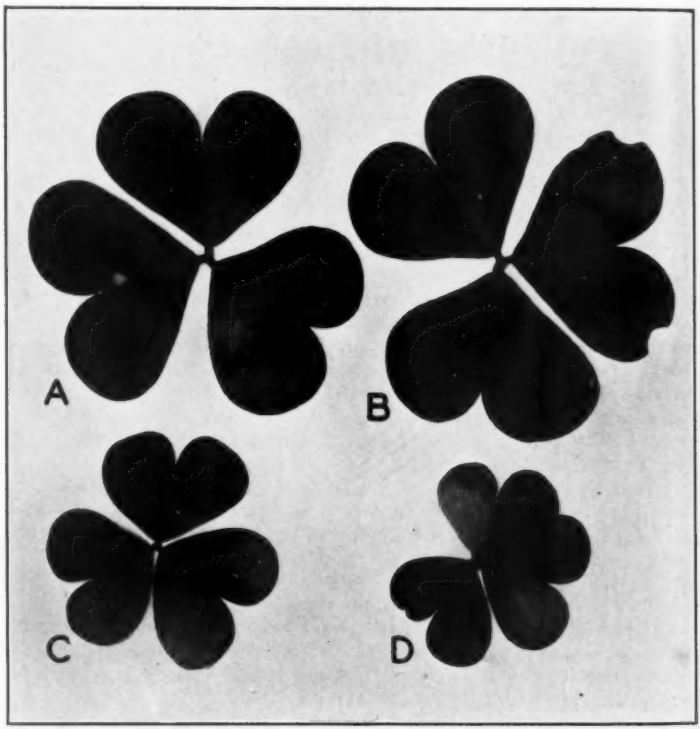


FIGURE 1.—*Puccinia sorghi* on oxalis leaves: *A*, Upper, and *B*, lower surface of leaf showing haploid infections 25 days old; *C*, upper, and *D*, lower surface of leaf bearing haploid infections 34 days old.

numerous spermogonia of an infection flow together into a single drop of fluid. As an unfertilized infection ages and spreads radially, the older spermogonia at the center die but are replaced functionally by younger spermogonia in the fresh marginal growth. In figure 1, *A*, *B*, are photographs of 25-day unfertilized infections and in figure 1, *C* and *D*, are 34-day infections. A few active spermogonia (marked by little drops of exudate) are still present in the margins of these infections. Production of spermogonial exudate continues in these

haploid, unfertilized infections as long as they live. The most extreme instance noted was a plant examined 43 days after inoculation. Only 2 or 3 of the numerous sterile infections remained alive but these still bore drops of exudate. This contrasts sharply with spermogonial behavior after fertilization. Whether fertilization comes early or late in the history of an infection, the surface dries in 2 or 3 days and no more exudate is formed. In fact, drying is the first indication (as seen with the naked eye) that fertilization has taken place.

When spermogonia begin to develop (about a week after inoculation), other hyphae grow outward to both the upper and lower leaf surface. In other cereal rusts studied, hyphae have been found growing out between ordinary epidermal cells. These are rare in *Puccinia sorghi* and, so far as noted, occur only on very young, tender oxalis leaves. Plate 2, *A*, from a 7-day infection, shows the hypha, *a*, attempting to force a passageway out through the upper epidermis. Such attempts are rarely successful.

More common are attempts to reach the surface at the stomata of both the upper and lower epidermis. Under ordinary conditions, stomatal hyphae are short-lived, for they are limited by the daily stomatal movements. In plate 2, *B*, from a 9-day infection, the hypha, *b*, pushed out between the guard cells, *a*, *d*, but was pinched and killed when the stoma shut. When the stoma reopened, a second hypha, *c*, pushed out alongside the first. This process may be repeated several times until the stoma is forced wide open and loses all motility. Examples such as plate 2, *C*, with the guard cells, *a*, *c*, wide apart and not less than a dozen hyphae, *b*, now dead, in the stomatal aperture, are fairly common in older, sterile infections.

When the stomata are closed, hyphae are apt to push out at the side or at the end of the guard cells. In plate 2, *D*, the stoma, *b*, is shut tightly, but hyphae, *a*, *c*, at both sides of the guard cells are working their way up to the upper surface.

In young isolated infections, haploid aecia form, undergo a limited development, and then deteriorate without forming spores. The time when aecial anlagen first appear in an infection varies with the conditions under which it is grown. The earliest noted are in infections 7 days old. New aecial anlagen continue to appear in the marginal growth for some time so that in older infections all stages from the beginning to full-sized aecia and the later stages of deterioration can be found.

The haploid aecium begins as a small tangle of interwoven hyphae just above the lower epidermis of the leaf. It is commonly but not always in the air spaces above a stoma. Plate 2, *E*, shows a bit of the lower epidermis including the cross section of the end of a stoma at *b*. Above it is a small aggregation of hyphae. Two slender hyphae (*a*, *c*) are pushing down at the end of the guard cells.

Plate 2, *F*, shows a larger aecium from material fixed 45 minutes after spermatia had been transferred to the surface of the infection, but the aecium is still essentially haploid. It has become organized into an upper half, *a*, composed of slender hyphae with rich contents (corresponding to the sporogenous area) and a lower half, *b*, of larger vacuolate cells where the hyphae are beginning to disarticulate into separate cells (the "space-making" tissue). This aecium, as commonly happens, centers on a stoma, *c*, which is filled with hyphae.

Plate 2, *G*, shows a typical detail from the sporogenous area of a slightly older haploid aecium drawn at high magnification. The cells are short, often isodiametric, and each contains rich cytoplasm and a single, large, spherical or ellipsoidal nucleus, which in turn contains a delicate chromatin net and a large central spherical nucleole.

In the absence of fertilization these haploid aecia undergo very little further differentiation. The cells may increase in size and become closely pressed against each other; then they gradually become impoverished in content and die. The first-formed aecia are spent and dead in infections 14 days old; but for some time new aecia continue to form in the margin of the growing infection. In plate 2, *H*, is represented a typical spent haploid aecium from a 30-day infection. The cytoplasm of the cells is gone and only remnants of nuclei remain. The infection from which this was drawn contained 75 of these dead haploid aecia. They are so small that they do not raise the leaf surface into mounds, nor are they seen with the naked eye in living infections.

Rarely, and under conditions little understood, a haploid aecium attains greater size and development before degeneration sets in. In these aecia occur monstrous teratological, multinucleate cells (pl. 3, *A*, *B*, *C*, *D*) of most irregular form and indeterminate growth. These cells soon die and the aecium as a whole degenerates.

RESULTS OF GREENHOUSE TESTS

Puccinia sorghi is heterothallic. The haploid individual is unisexual and incapable of producing fertile aecia. The isolated infection to which no spermogonial exudate is brought from another and different infection grows old and dies without producing fertile aecia. Figure 1, *A*, *B*, already referred to, shows isolated infections 25 days old, and *C*, *D*, 34 days old. These old infections are still growing vegetatively and their spermogonia are still producing spermatia but they cannot form open aecia. Few infections live longer than this, as the oxalis leaves bearing them are fragile and comparatively short-lived. Only the most favorably situated infections live longer than 35 days. The bisexual or diploid mycelium, which leads to the formation of aeciospores, is initiated only when the two sexes meet. The two sexes can be brought together in several ways.

When two contiguous infections of opposite sex grow until their mycelia interlace within the leaf, open aecia are promptly produced (fig. 2, *B*). In rusts with abundant exudate it remains uncertain whether fertilization occurs here by anastomosis of hyphae within the leaf or by a flowing together of the two drops of spermogonial exudate on the leaf surface. In *Puccinia sorghi*, however, spermogonial exudate is produced in such small volume that ordinarily no large drop is formed on an infection; there is only the minute individual drop for each spermogonium. If undisturbed there is small chance that the exudates of the two infections will mix until the two are fully confluent. Moreover, the outermost zone of growth of any infection

EXPLANATORY LEGEND FOR PLATE 3

A, *B*, *C*, *D*.—Multinucleate cells from haploid aecium. ×1,400

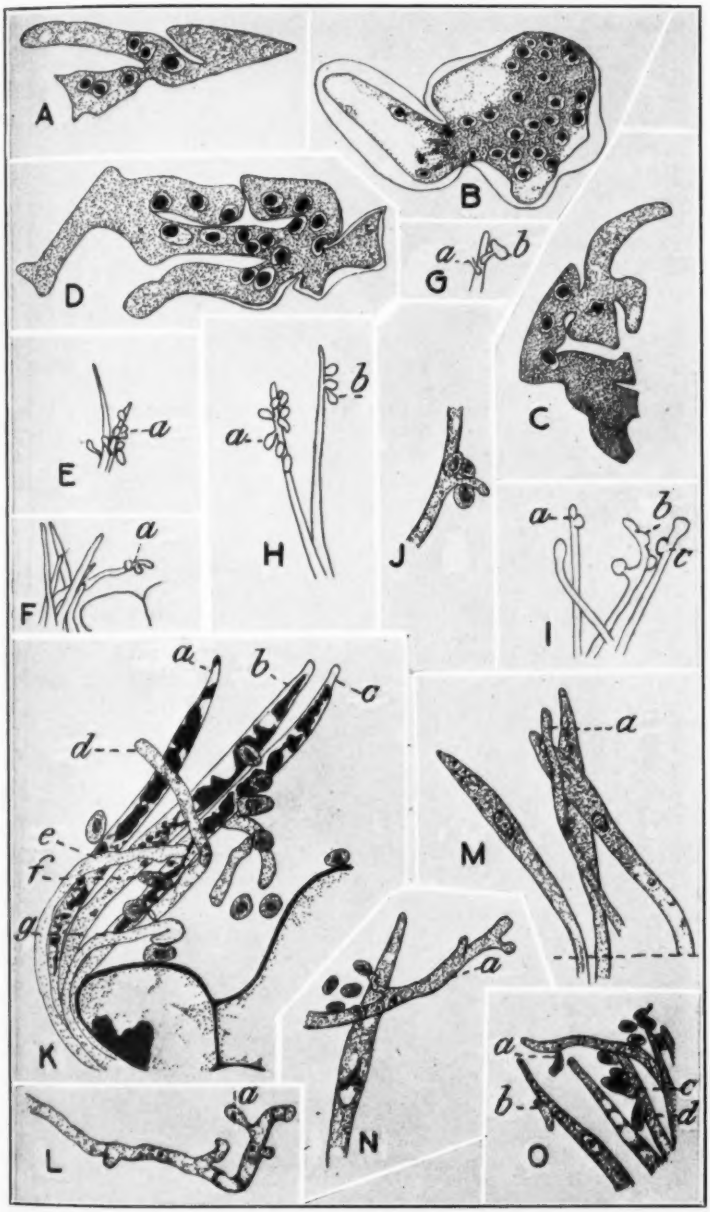
E, *F*, *G*, *H*, *I*.—Sketches from living material showing paraphyses with attached spermatia, *a*, *b*, *c*. Magnification not measured.

J.—Irregular branched paraphysis. ×1,020.

K.—Detail with decadent paraphyses, *a*, *b*, *c*, irregular secondary paraphyses, *d*, *e*, *g*, and spermatia, *f*. ×1,400.

L, *M*, *N*.—Irregular paraphyses at *a*. *L* and *M*, ×1,020; *N*, ×1,400.

O.—Paraphyses with spermatia, *a*, *b*, attached in fixed material. Spermatia, *c*, *d*, germinating. ×1,020.



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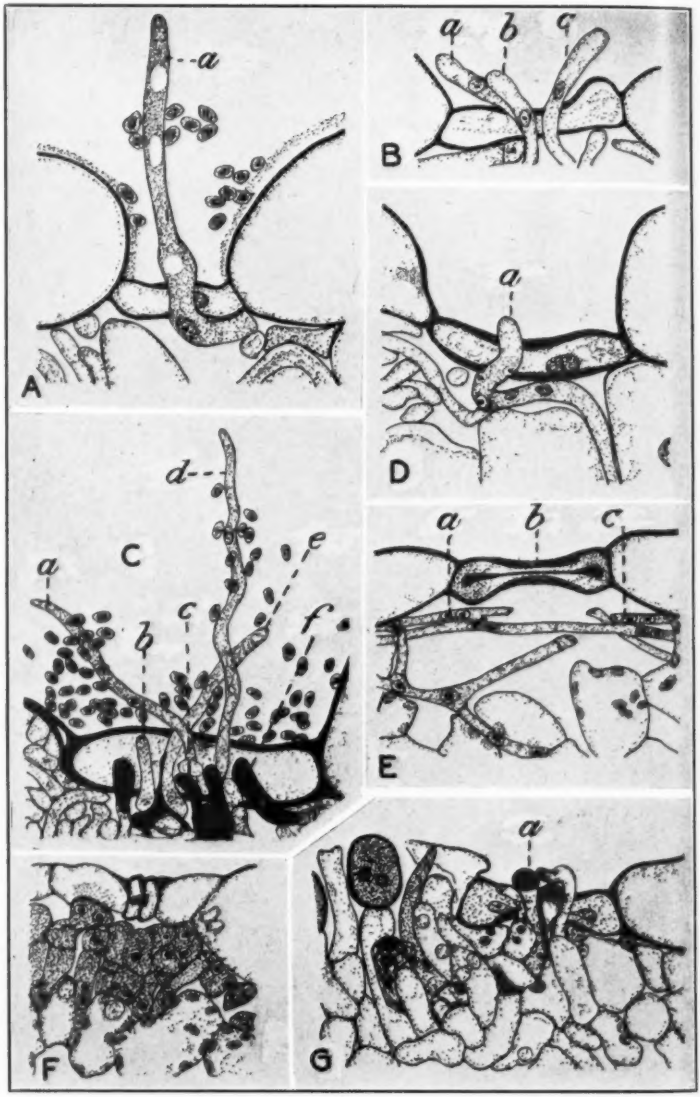

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consists of vegetative hyphae only. In other words, the hyphae would meet within the leaf a day or two before the exudates would meet on the surface of the leaf. It has often been noted in such cases that the surface of the infections dry (i. e., stop forming spermogonial exudate) very soon after the two infections become tangent. As before stated, this drying of the surface is the first macroscopic indication of the incidence of fertilization. It seems probable then

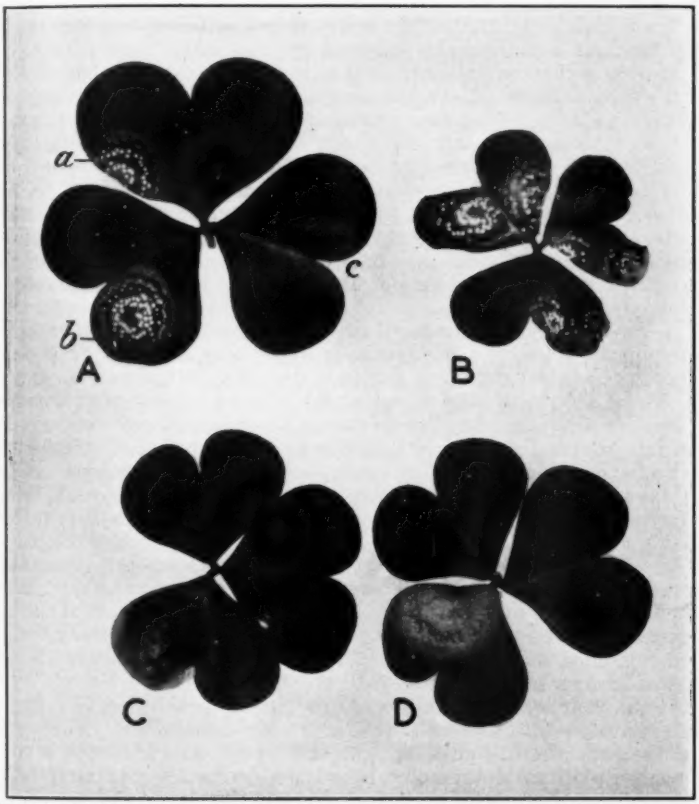


FIGURE 2.—*Puccinia sorghi* on oxalis leaves: *A*, Leaf with three 25-day infections, 2 fertile, *a*, *b*, spermatized when 10 days old, and 1 sterile, *c*; *B*, overlapping infections; *C*, upper, and *D*, lower surface of leaf bearing infection fertilized when 28 days old and photographed when 37 days old.

although, of course, not proved, that diploidization in *P. sorghi* can be effected by means of mycelial anastomoses. In the great majority of cases, however, the two sexes are brought together by the transfer of spermatia from one infection to another. This may be brought about by direct contact between the infections, transfer by insects, or artificially in controlled experiments.

EXPLANATORY LEGEND FOR PLATE 4

A, *B*, *C*.—Stomatal hyphae at *a*, *b*, *c*, *d*, *e*, *f*, from infections in damp chamber. ×1,020.
D.—Stomatal hypha, *a*, containing several nuclei. ×1,020.
E, *F*, *G*.—Details from uredial infections: *E*, vegetative hyphae, *a*, *c*, beneath stoma, *b*; *F*, young uredium; *G*, detail of uredium from infection in damp chamber, with young spores pushing out through stoma at *a*. ×640.

In several of the earlier experiments a larger percentage of infections developed aecia than would theoretically be expected. It was noted repeatedly that when an oxalis leaf bore a single infection and was left undisturbed the infection remained haploid and sterile; but if several infections occurred on one leaf, whether some distance apart on the same leaflet or even on different leaflets, a certain percentage developed aecia. In these experiments efforts were made, of course, to exclude insects whose successive visits to two or more infections could result in transferred spermatia. In spite of precautions, however, a few fertilizations were probably effected by insects.

More were probably due to the motions of the oxalis leaflets. Each of the three leaflets of the compound leaf of oxalis has a motor organ in its short petiolule whose action bends the leaflet down against the main petiole. Moreover, each leaflet can fold up along its midrib. This motility of the leaflets might bring into contact the surface of two infections located in corresponding positions in the two halves of a leaflet or two infections in juxtaposed halves of adjoining leaflets. Just how much interchange of spermatial exudate occurs through the direct contact between two infections effected by these photeolic movements of the leaflets is not known, but it probably is a factor of some importance.

In controlled experiments spermatia are transferred by means of a fine pipette, a brush, a toothpick, or by picking one infected leaf and applying its infected surface gently to that of an attached leaf so that spermatia are transferred directly and without contact with any tool.

Results show that fertilization takes place equally well whether the exudate be transferred to the upper or the lower surface of an infection. In one experiment a leaf bore seven well-scattered infections. When 10 days old, exudate from several infections was transferred to the upper surface of these seven infections. Six days later all seven bore open aecia.

Another leaf bore three 10-day infections, and exudate was transferred to the lower surface. Two of the three developed aecia. Later, when these three infections were 25 days old, the underside of the leaf was photographed (fig. 2, *A*). It is to be inferred that the infection that remained sterile (fig. 2, *A*, *c*) either received no exudate or only that of its own sex.

At the time when exudate was brought to these infections (fig. 2, *A*, *a*, *b*, *c*) the latter were only 1 or 2 mm in diameter. They grew rapidly and, when 16 days old, the two fertile ones produced a circle of aecia 4 or 5 mm in diameter. As the mycelium spread marginally, a second outer circle of aecia formed, and still later a third (fig. 2, *A*, *a*, *b*). In other words, a single fertilization, when infections are only 1 or 2 mm in diameter, suffices for the production of fertile aecia as long as the infection lives, and the outer circles of aecia form in territory far beyond the area occupied by the fungus at the time of fertilization. This is possible only if the mycelium is diploidized as a consequence of fertilization and if the diploid mycelium grows marginally and initiates successive circles of new fertile aecia there. The sterile infection (fig. 2, *A*, *c*) made an equally good vegetative growth (it is of the same diameter as *a* and *b*) but it has produced no open aecia.

The question arises as to how early and how late in the history of an infection fertilization can take place. Spermogonial exudate was transferred to infections of different ages. In some experiments the

exudate was placed on the upper leaf surface and in others on the lower surface. Fertilization was successful in infections 10, 14, 28, 35, and 36 days old, whether the spermatia were applied to the upper or to the lower surface of the leaf. In the younger infections the leaf dried (i. e., the spermogonia stopped producing spermatia) 2 days after fertilization, and open aecia appeared on the sixth day. In older infections the leaf dried in 3 days and aecia opened usually on the seventh or eighth day.

The distribution of the fertile aecia in infections fertilized when young is different from that in infections fertilized in old age. In one experiment exudate was transferred to the upper surface of three sterile infections 28 days old. They were about 7 mm in diameter at that time. All developed open aecia. Two of these were photographed when 37 days old, one (fig. 2, *C*) showing the upper surface and the other (fig. 2, *D*) the lower surface. The aecia have formed (fig. 2, *D*) in a marginal circle. This contrasts sharply with the distribution of aecia when fertilization takes place at 10 days (fig. 2, *A*) and indicates that, while an infection is capable of effective fertilization as long as it lives, aecia will develop and open only in the young marginal growth. As stated earlier (p. 1051), small haploid aecia start forming in an infection when it is 1 week old (about 2 mm in diameter) and continue to form in new marginal growth of the rust as long as it lives. Figure 2, *D*, proves, however, that a given haploid aecium soon deteriorates and loses the power to be stimulated into normal development, for none of the haploid aecia in the older central area of the infection has developed.

Ordinarily one circle of aecia is all that can be expected in cases of greatly delayed fertilization. Rarely, still further growth takes place; for example, in one experiment four exceptionally vigorous infections 35 days old were fertilized. On the forty-second day a marginal circle of aecia opened, and on the forty-sixth day a second outer circle of young aecia was forming.

ENTRANCE OF SPERMATIAL NUCLEI

Transferring spermatia of one sex to the surface of an infection of opposite sex results in the diploidizing of the mycelium and the production of fertile aecia. Attempts have been made to determine the way or ways in which this takes place. For this purpose pots of oxalis plants were inoculated, and when the infections matured the spermogonial exudate was transferred back and forth among the infections, the attempt being made to bring exudate to each infection from several others. Then the plants were placed in a damp chamber. Material was removed at short intervals, part of which was fixed and the rest used for making freehand sections of the living material.

Evidence was presented in a brief preliminary paper (*6*) that fertilization in corn rust can take place by fusion of spermatia with fresh young paraphyses of the spermogonia. The spermatium becomes joined to the paraphysis, either directly or through a short connecting hypha, and the spermatial nucleus passes over into the paraphysis.

The figures formerly given are not reproduced here. Additional sketches, however (pl. 3, *E*, *F*, *G*, *H*, *I*), from freehand sections of the living material show the external part of the paraphyses with spermatia attached. The spermatium may be attached directly as shown in living material in plate 3, *F*, *a*; *I*, *a*, *b*, *c*, and in fixed material in

plate 3, *O*, *a*, *b*, or through a short connecting hypha as shown in plate 3, *E*, *a*; *G*, *a*, *b*; *H*, *a*, *b*.

The period during which a given paraphysis can serve to receive spermatia is probably short, for paraphyses, like any other rust hyphae exposed to the outer air, deteriorate rapidly. In plate 3, *M*, is shown the appearance, in fixed and stained material, of a group of fresh paraphyses, and in plate 3, *K*, *a*, *b*, *c*, is shown a tuft of dead paraphyses.

A spermogonium, however, continues to produce new paraphyses for a considerable period. When the plant bearing the infections is placed in a damp chamber and new paraphyses emerge into saturated air, they tend to grow more like the ordinary hyphae of the interior of the leaf. They remain slender and thin-walled and do not taper to a point (pl. 3, *F*, *a*; *M*, *a*; *O*, *a*) and they follow an irregular course and may even branch (pl. 3, *J*; *L*; *N*, *a*). These irregular paraphyses, like the regular ones, serve to receive spermatial nuclei (pl. 3, *F*, *a*; *L*, *a*; *O*, *a*).

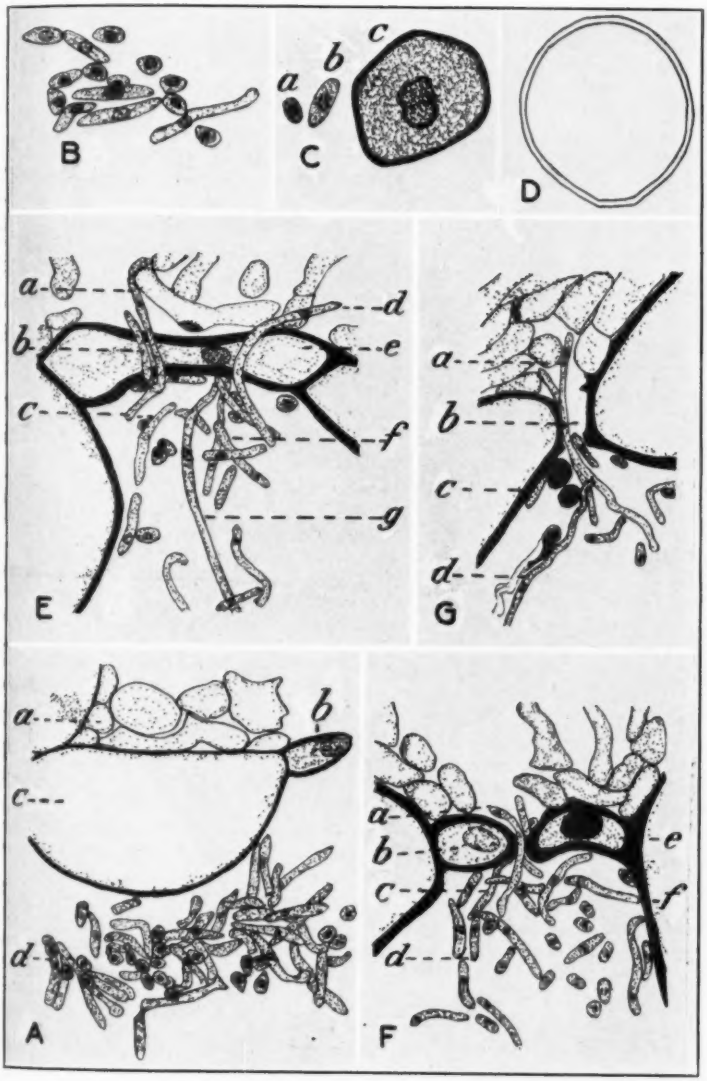
Spermatial nuclei commonly enter the haploid mycelium via the paraphyses. There are indications, however, that spermogonia are not the only ports of entry. Reference has been made to the stomatal hyphae, and examples have been figured (pl. 2, *B*, *C*). Stomatal hyphae are not nearly so abundant in corn rust as in other rusts studied, but are regularly present in small numbers, especially in stomata adjoining spermogonia and young aecia. Under ordinary conditions these stomatal hyphae remain short, scarcely projecting beyond the guard cells, and soon are killed by the closing of the stoma. When, on the contrary, the infected plant is placed in a damp chamber, the stomata open wide and the stomatal hyphae grow out freely and may attain considerable length (pl. 4, *A*, *a*; *B*, *a*, *b*, *c*). In plate 4, *C*, there are a number of old dead stomatal hyphae, at *c* and *f*, of the ordinary type, and four (*a*, *b*, *d*, *e*) that formed after the plant was put into the damp chamber.

It is probable that these stomatal hyphae also serve to receive spermatial nuclei, although the actual entrance of spermatial nuclei into stomatal hyphae has not been observed. That it does occur, however, is evidenced by the presence of several nuclei in the hypha at plate 4, *D*, *a*, and in a number of similar figures of corn rust by Rice (*44*).

It may be suggested in opposition to this idea that in any heavily infected leaf tissues vegetative hyphae radiate out in all directions through intercellular spaces in search of fresh food supplies and that such hyphae might enter stomatal apertures just as they would enter any other intercellular space. If the entrance of such hyphae is purely fortuitous and has no sexual significance, it should be found quite as frequently in sporophytic as in gametophytic mycelium. To determine this, uredial infections of *Puccinia sorghi*, 5, 7, 9, 12, 15, and 20 days old, growing on corn, were fixed, embedded, and sectioned.

EXPLANATORY LEGEND FOR PLATE 5

A.—Germinating spermatia, *d*, on lower epidermis, *b*, *c*, with aecial cells above at *a*. ×1,020.
B.—Germinating spermatia. ×1,400.
C, *D*.—Relative size of spermatium, *a*, germinating spermatium, *b*, aeciospore, *c*, and urediospore, *D*. ×1,400.
E.—Stoma, *e*, with germinated spermatia, *a*, *b*, *d*, entering, and others, *c*, *f*, *g*, growing toward stoma. ×1,400.
F.—Stoma, *b*, *e*, with germinating spermatia, *c*, *d*, *f*, outside, and others, *a*, entering. ×1,400.
G.—Break in epidermis, *b*, with germinating spermatia, *c*, *d*, outside, and others, *a*, entering. ×1,400.



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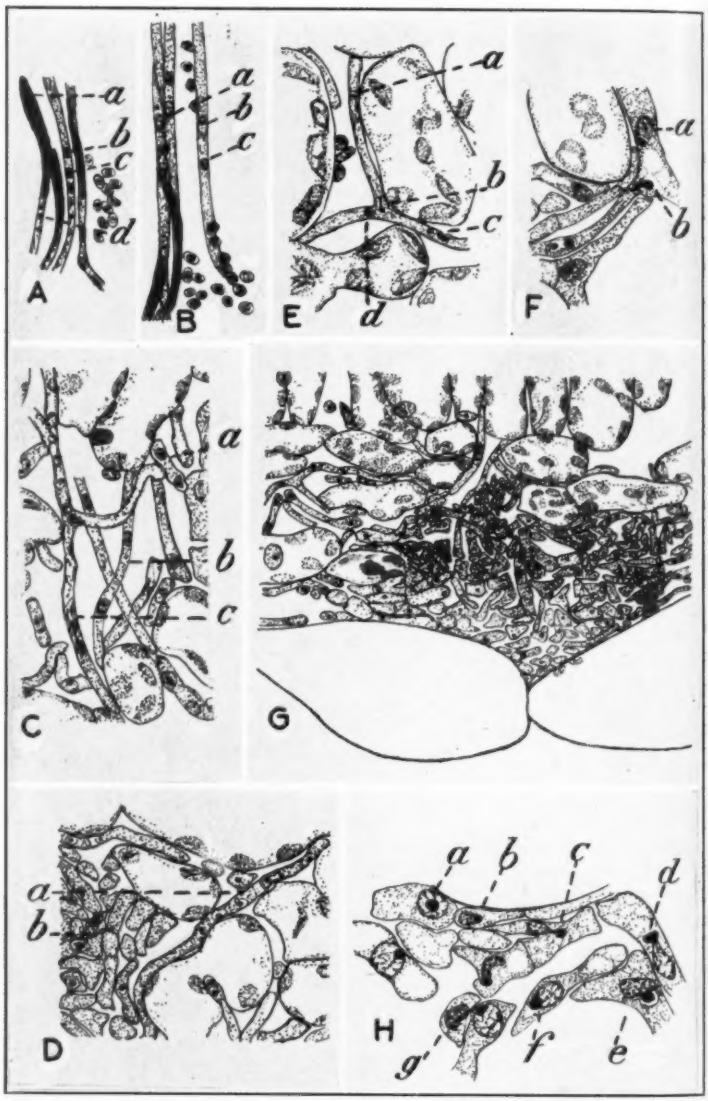

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In a section-by-section survey of several infections of each age, not a single hypha was found inserted into a stomatal aperture. These vegetative diploid hyphae frequently enter the substomatal air chamber but commonly grow straight across it (pl. 4, *E*, *a*, *c*), without entering the stoma, *b*. With the onset of reproductive activity, hyphae grow out to both the upper and lower epidermis and branch richly there in preparation for the formation of urediospores. A continuous area of subepidermal mycelium is formed, which ordinarily includes several substomatal air chambers. Search here failed to reveal a single stomatal hypha (pl. 4, *F*). Later, the growing uredium lifts, stretches, and flattens the epidermis and eventually breaks it. As the uredium is about to open, one spore and its stalk may be thrust through the weakened epidermis slightly in advance of the general opening, but this never, so far as noted, takes place at a stoma.

As a further test, a pot of corn seedlings bearing uredial infections was placed in a damp chamber before fixing the material. In 100-percent humidity the stomata open wide. Even here, no hyphae enter stomata save at the margins of open uredia where the young spores on their elongating stalks are pushing out (pl. 4, *G*, a).

Since stomatal hyphae are limited to the haploid generation and are most abundant here at the time when fertilization is due and are, in the main, affiliated with the reproductive structures (spermogonia and haploid aecia), the inference is that their formation is not accidental but that they function in reproduction.

There is evidence of still another method of fertilization. When spermatia have been transferred to an infection and the infected plant has been placed in air saturated with moisture, the spermatia germinate. Germinating spermatia have been found in material grown in the greenhouse in 1932 and in that grown out of doors on a balcony in 1933. In plate 5, *A*, drawn at high magnification, is shown a mat of such germinating spermatia, *d*, on the lower epidermis, *b*, *c*, of the leaf adjoining an aecium of which a few cells are represented at *a*. In plate 5, *B*, is shown a group of germinating spermatia still more highly magnified. In germinating, the spermatium swells, elongates first to an ellipsoid, then to a cigar shape, and then to a longer hypha. So far as noted it remains thin-walled, nonseptate, and uninucleate, and has but a scant supply of cytoplasm. Apparently, only the spermatia on the lower epidermis of the leaf germinate—they are more protected there.

A comparison of the germinating spermatia (pl. 5, *A*, *d*) with the fungus cells of the aecium just inside the epidermis at *a* gives an idea of the minuteness of these germinated spermatia. A further comparison of relative sizes is given in plate 5, *C* and *D*. Plate 5, *C*, *a*, represents an ungerminated spermatium; plate 5, *C*, *b*, an early stage of germination; *C*, *c*, represents an aeciospore of the same rust; and plate 5, *D*, a urediospore, all at the same magnification (× 1,400).

EXPLANATORY LEGEND FOR PLATE 6

A.—Paraphyses at level of ostiole. Decadent paraphysis, *a*, *d*. At *b*, *c*, may be spermatial nuclei in paraphysis. ×1,020.

B.—Detail of paraphyses at level of ostiole. At *a*, *b*, *c*, may be spermatial nuclei inside of paraphysis. × 1,020.

C.—Mycelial cells with more than 1 nucleus at *a*, *b*, *c*. ×1,020.

D.—Multinucleate mycelial cell, *a*, at edge of aecium, *b*. ×1,020.

E.—Detail of mycelium with septum at *a*, a dividing nucleus at *b*, a pair of nuclei at *c*, and a resting nucleus at *d*. ×1,020.

F.—Detail of mycelium with migrating nucleus at *a*, and at *b* nucleus passing through wall. ×1,400.

G.—Diploidization of young aecium. ×460.

H.—Detail of aecium in early stage of diploidization, with quiescent nucleus, *a*, and active nuclei, *b*, *c*, *d*, *e*, *f*, *g*. ×1,400.

The spermatial hyphae are so delicate and possess so little of stainable content that only a strong, sharp stain makes them visible.

There is evidence that spermatial hyphae can enter the host leaf. In plate 5, *E*, *e*, is a stoma in longitudinal section. Three spermatial hyphae, *a*, *b*, *d*, have grown up between the guard cells into the substomatal chamber and others, *c*, *f*, *g*, are growing in that direction. In plate 5, *F*, is drawn a cross section of a stoma, *b*, *e*, through which three spermatial hyphae at *a* have entered the leaf, while others outside, at *c*, *d*, *f*, are growing toward it. In plate 5, *G*, at *b*, is a small accidental break in the epidermis caused by the tension from a growing spermogonium above it. Two spermatial hyphae have grown through the crack and made contact with spermogonial cells inside at *a*, while others outside, at *c* and *d*, are growing toward the opening. While proof is lacking that these spermatial hyphae anastomose with the haploid mycelium inside the leaf and contribute their nuclei for its diploidization, it seems probable that this takes place.

An unsuccessful attempt was made to determine whether spermatia placed on a spermogonium may also germinate and grow down through the ostiole. When paraphyses are young and fresh a spermatium becomes attached directly to a paraphysis and contributes its nucleus to it (pl. 3, *O*, *a*, *b*). But when paraphyses are old and decadent, as in plate 3, *K*, *a*, *b*, *c*, it would serve a useful purpose if the spermatia could grow into the spermogonium and make contact there with living hyphae. Early stages of spermatial germination are readily found among paraphyses (pl. 3, *O*, *c*, *d*; *K*, *f*). Later stages, if they occur, are hard to recognize. It may be that among the slender hyphae about a decadent spermogonium (pl. 3, *K*, *d*, *e*, *g*), which have been assumed to be secondary paraphyses growing outward through the ostiole, are some overlooked examples of germinating spermatia growing inward. This remains unproved.

DIPLOIDIZATION OF MYCELIUM AND AECIA

Spermatial nuclei that succeed in entering haploid hyphae multiply and spread rapidly from the point or points of entrance. As stated earlier, a count of 100 cells in an unfertilized infection gave 94 uninucleate and 6 binucleate cells. On the other hand, within 24 hours after spermatia were transferred to the surface of an infection, cells with more than one nucleus were found from one end of the mycleium to the other. A count of 100 cells in a 13-day infection 24 hours after spermatia were placed on the surface gave 40 cells with 1 nucleus, 37 with 2, 19 with 3, and 4 with 4 nuclei. A 60-percent diploidization in 24 hours could hardly be achieved in any other way than by nuclear migrations through hyphae already formed.

An attempt has been made to learn something about this process of diploidization. Efforts to trace the progress of spermatial nuclei from the point of entrance into a paraphysis down through the ostiole and out through the wall of the spermogonium were unsuccessful. Drawings *D* and *E* of plate 1 show the ostiole of a spermogonium densely crowded with paraphyses and spermatia, and show how difficult it would be to be sure whether, for instance, the small bodies in plate 1, *E*, *b*, *c*, are incoming spermatial nuclei traveling down inside of a paraphysis or merely spermatia of the native spermatial exudate coming out through the ostiole. Plate 6, *A* and *B*, shows details of paraphyses at the level of the ostiole. In plate 6, *A*, *b*, *c*, and *B*, *a*, *b*, *c*, the small stained bodies are quite certainly inside the paraphyses,

but here another doubt arises. Paraphyses are short-lived and as their living content dies it takes a dense stain. Dead cytoplasm (pl. 6, *A*, *a*) stains a solid black, but in the early stages of this deterioration small dark-stained patches occur in the cytoplasm (pl. 6, *A*, *d*) that are sometimes misleading. The probability is, however, that the small bodies shown in plate 6, *A*, *b*, *c*; *B*, *a*, *b*, *c*, are spermatial nuclei migrating down inside of paraphyses.

The later distribution of spermatial nuclei and their progeny can be more easily followed. Cells with two or more nuclei are soon widespread in the mycelium. In plate 6, *D*, a hypha, *a*, leading to the edge of an aecium, *b*, contains at least six nuclei. Plate 6, *C*, shows mycelial binucleate cells at *a* and *b*, while at *c* is a hypha with six nuclei and no visible septum between them. Septa here are thin and delicate, and the end of a cell is more often marked by a slight break in the cytoplasm (pl. 6, *E*, *a*) than by any sharply stained cross wall. Thin spots would facilitate greatly the passage of a nucleus along a hypha. Proof that a nucleus can pass through from one cell to another is seen in plate 6, *F*, *b*. The detail drawn in plate 6, *E*, found in marginal mycelium, suggests a process of diploidization like that found in the Agaricales. At *c* is a pair of nuclei consisting of 1 large and 1 small nucleus. At *d* is a resting nucleus and beside it, at *b*, a small dividing nucleus in telophase. A possible interpretation here is that a small migrating nucleus of spermatial origin divided, leaving behind one daughter nucleus at *c*, while the other moved on and divided again at *d*. On this basis, one of the daughter nuclei at *b* would again move on to the next cell. By such successive divisions and migrations mycelium traversed by spermatial nuclei would be diploidized.

When nuclei of spermatial origin reach a fresh young haploid aecium, it, too, becomes rapidly diploidized. This may take place to a small extent by ingrowth of diploid hyphae from the mycelium outside the aecium, but probably is principally due in corn rust to the migration of nuclei through existing hyphae into the aecium. A count of 100 cells in marginal and sporogenous areas of aecia 24 hours after spermatization gave 68 cells with 1 nucleus, 26 with 2, 5 with 3, and 1 with 4 nuclei. Plate 6, *G*, represents one of these aecia. Cells with more than one nucleus are to be found in all parts of the sporogenous and marginal areas.

The cells and their nuclei are larger and more favorable for study in the aecium than in the mycelium. Plate 6, *H*, shows a detail at high magnification. A comparison of this with a corresponding bit of a haploid aecium of the same age (pl. 2, *G*) and a detail from a young spermogonium (pl. 1, *C*) shows one striking difference. In the haploid structures the spherical nucleus of each resting cell is placed in the center of the cell, and its nucleole is in the center of the nucleus. Here (pl. 6, *H*), on the contrary, there is evidence of unusual activity. An occasional nucleus (pl. 6, *H*, *a*) still has the appearance of quiescence, but more of them (pl. 6, *H*, *b*, *c*, *d*, *f*, *g*) show a more or less elongated sack shape with the nucleole at the narrow end of the sack. The appearance suggests that at the moment of fixation the nuclei were moving about and that the nucleole leads when a nucleus travels. Apparently not only the introduced nuclei of spermatial origin move, but the larger, native nuclei are also active. The mechanism of this nuclear locomotion, whether ciliary or amoeboid, is unknown. No cilialike structures or polar radiations have been observed. A pos-

sible hint as to the means of locomotion is shown in the small body near the nucleus in plate 7, *D*, at *a*. It is interesting that this group of cells (pl. 6, *H*), although showing decided evidence of stimulation, is still haploid. Only one cell (pl. 6, *H*, *e*) contains a small nucleus (presumably of spermatial origin) in addition to the large nucleus.

Further details from young aecia in process of diploidization are given in plate 7, *A* to *F*. In *A*, the small nucleus at *b* would seem to be about to move through the septum to join the larger nucleus at *c*; in *E*, *a*, the nucleus is passing through the septum; in *C* are three cells, each with a large elongated nucleus close to a small nucleus; and in *B* at *a* and *b* are closely associated pairs of nuclei, each consisting of a large and a small nucleus. Although nuclei become paired at this time, no nuclear fusions occur, so far as noted. At *F*, *a*, *b*, are suggestions of nuclear divisions, *F*, *a*, probably being a division of a spermatial nucleus, and *F*, *b*, one of the earliest conjugate divisions; at *D*, *c*, and *A*, *a*, are pairs of young daughter nuclei.

Plate 7, *G*, represents a later stage in the development of the aecium found in material fixed 2 days after placing spermatia on the leaf surface. The aecium has grown (pl. 6, *G*) and definite progress toward spore formation is evident. The great majority of the cells in the sporogenous area are binucleate, although an occasional uninucleate cell can still be found. The rapid migrations and divisions of the nuclei derived from spermatia are nearing completion. The two nuclei of a cell, at first so unequal, have now grown to equal size. It is no longer possible to distingusih the progeny of spermatial nuclei from those of the original haploid mycelium; both are spherical or ellipsoidal and contain a large central nucleole. At this time (pl. 7, *G*) the young "basal" cells are growing toward a common center and the first conjugate divisions of the nuclei have taken place. A few aeciospore initials have formed.

In later development each of these basal cells gives rise to a chain of aeciospores and as the aecium grows it presses upon the lower epidermis of the leaf and finally ruptures it, setting free the aeciospores. A detail from a growing aecium, showing the base of several spore chains, is shown in plate 7, *H*. The closely crowded basal cells form a layer from *c* to *f*. Some of them have short bases and others have grown down from cells at a higher level, *e*, *b*. A few binucleate cells higher up, as at *a*, will probably still grow down to form new basal cells interpolated between those already formed. Additional spore chains also may be formed by the branching of basal cells, as at *d*. No evidence has been noted of fusion of two uninucleate cells to form a binucleate basal cell.

DISCUSSION

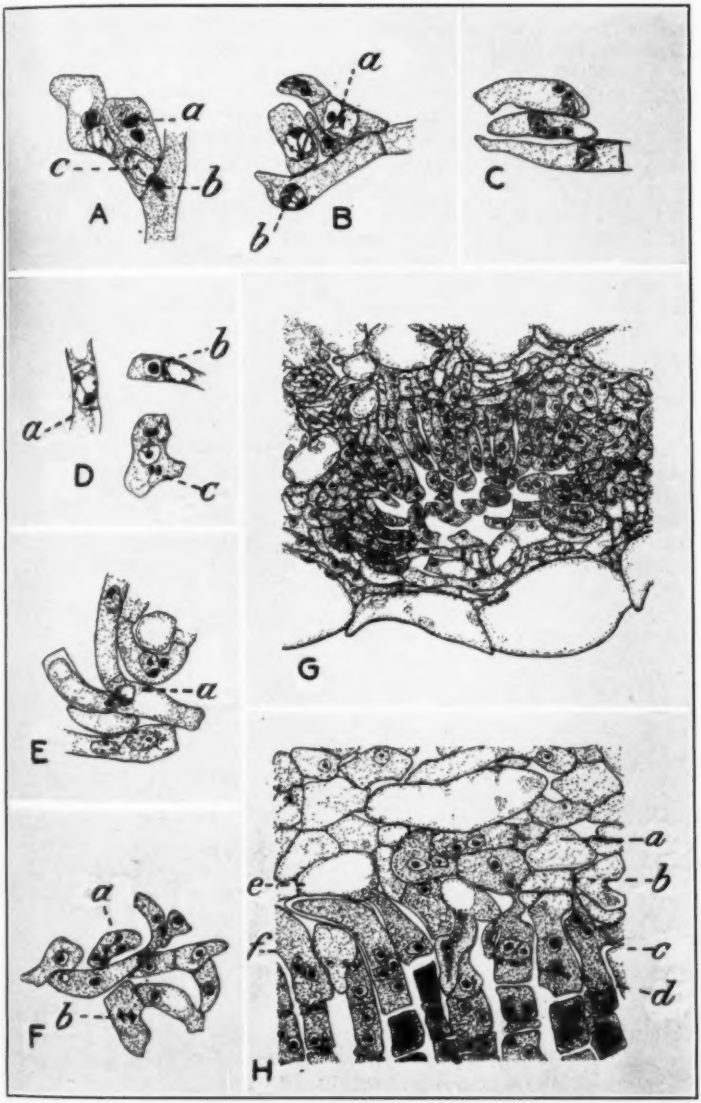
The formation of haploid aecial anlagen by the haploid mycelia of rusts is of general occurrence, but the degree of development attained by these aecia in the absence of fertilization varies greatly in different rusts. At one extreme of the series is flax rust (*5*, *8*), in

EXPLANATORY LEGEND FOR PLATE 7

A to *F*.—Details showing diploidization of aecium, with dividing nuclei at *F*, *a*, *b*; newly divided nuclei at *A*, *a*, and *D*, *c*; small nucleus about to pass wall at *A*, *b*, *c*, and one passing through at *E*, *a*; paired nuclei at *B*, *a*, *b*, *C*, and *D*, *b*; and nucleus attended by small granule at *D*, *a*. ×1,400.

G.—Aecium 2 days after spermatization. ×460.

H.—Detail from older fertile aecium, showing spore chains, *c*, *d*, *f*, and the cells, *a*, *b*, *e*, which give rise to them. ×1,020.



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which even the occurrence of haploid aecia is doubtful. The ring of subepidermal pseudoparenchyma about each spermogonium may perhaps be interpreted as an aecial anlage although it possesses no organization remotely suggesting an aecium. In corn rust numerous small haploid aecia form and rapidly die. Rarely a few multinucleate cells form in these aecia before degeneration sets in. In *Puccinia coronata* (*4*) and *Uromyces* (*9, 10*) the haploid aecia are larger and they regularly develop multinucleate cells before deteriorating. At the other extreme are a few rusts like the strain of *Caeoma nitens* Schw., described by Dodge (*27*), in which haploid aecia can complete development and produce haploid aeciospores.

Each addition to the literature of heterothallism in rusts makes it clearer that a rust may use any of several methods to achieve the bisexual condition necessary for the normal development of fertile aecia.

It is probable that when rust mycelia of opposite sex meet in the tissues of a leaf the hyphae anastomose and an interchange of nuclei takes place. It is difficult to prove this by direct observation, for in the irregular air passages of the mesophyll tissue it is not possible to trace any given hypha to its source. Direct proof that anastomoses occur in rusts, however, is furnished by *Puccinia triticina* (*1*). In infection by urediospores, germ tubes from two spores may form appressoria on the same stoma and unite there or may enter the stoma and fuse just after entry. This makes possible the bringing together of nuclei of different parentage and could conceivably be of great service to a rust like *P. triticina*, often growing for a prolonged period in the absence of the aecial host.

Indirect but none the less conclusive evidence of mycelial anastomoses followed by diploidization of haploid mycelium is reported by Brown (*15*). In *Puccinia helianthi*, an autoecious rust, if uredial (diploid) infections are grown alongside of haploid infections and the mycelia interlace, the haploid mycelium at once produces fertile aecia. Evidently the diploid hyphae anastomose with the haploid hyphae and contribute to them nuclei of the sex they lack. A corresponding diploidization of a haploid mycelium by a diploid mycelium is known in the Agaricales (*16, 17*).

It may be that this interchange of nuclei through mycelial anastomoses serves in another group of rusts. Microcyclic rusts without spermogonia are supposedly homothallic and without any means of bringing together nuclei of different parents. It may be found, however, that when by chance two haploid mycelia of such a rust interlace, an active interchange of nuclei takes place. In the great majority of cases in rusts, however, the bisexual mycelium is established through the agency of spermatia. This, too, may take place in several ways.

Evidence is accumulating that in some rusts the paraphyses serve as ports of entry for spermatial nuclei. Earlier work on *Puccinia graminis* (*2, 7*) showed that soon after spermatia were placed on the surface of an infection cells in the walls of the spermogonia and in hyphae leading from them were frequently multinucleate, and the inference was that spermatial nuclei had entered the paraphyses and passed down through them to the wall of the spermogonium. Recently, Craigie (*25*) has published a photograph showing "the union of a pycniospore and a flexuous hypha (found among the paraphyses of a spermogonium) in a haploid pustule of *P. helianthi*."

Pierson (*42*), working with *Cronartium ribicola*, in which the spermatiophores are arranged in an extensive compact crust, found that an occasional hypha projects beyond the level of the crust and that, when spermatia are brought from other infections, these longer, exposed hyphae receive the spermatial nuclei. In corn rust (*6*) the paraphyses of the spermogonia serve as receptive hyphae and details of the transfer of spermatial nuclei to paraphyses have been found.

There is nearly as much evidence that stomatal hyphae and hyphae that push out between ordinary epidermal cells can function as receptive hyphae. Andrus (*9*, *10*), working on species of *Uromyces*, believes that trichogynes grow out from "eggs" in the aecial primordia to the leaf surface "where they project through stomata or between epidermal cells and fuse with spermatia transferred by insects, by hand or by other agencies." In *Puccinia triticina* (*3*) and *P. coronata* (*4*), receptive hyphae grow into stomatal apertures and force a passageway out to the leaf surface between epidermal cells; soon after spermatization these receptive hyphae are found crowded with extra nuclei, presumably of spermatial origin. In *P. sorghi* it is found that stomatal hyphae occur only in the haploid generation, that they are prominent at the time when fertilization is due, and that they are usually associated with reproductive structures. The entrance of spermatial nuclei into stomatal hyphae has not been seen in corn rust, but directly after spermatization (*44*, and the present paper) these surface hyphae are found to contain extra nuclei.

It is probable, too, that under favorable conditions spermatia can function in yet another way to bring about the bisexual condition. Ever since the early days of rust research it has been an open question whether spermatia can germinate and, if they can, whether their germination serves any purpose. Cornu (*21*), in 1876, presented evidence that spermatia can germinate by budding, i. e., they can "émettre des sporidies secondaires" and so decided that "Les spermaties et les urédospores sont de simples conidies." Plowright (*43*), in 1889, also obtained a yeastlike budding of rust spermatia in honey. He states that this budding "points rather to their being conidia than spermatia." Carleton (*18*), in 1893, likewise found budding of spermatia but considered it as merely stunted growth; later he (*19*) obtained normal germ tubes from spermatia. All efforts to produce infections by means of spermatia failed, however, and interest in the question lapsed.

With the discovery of heterothallism in rusts the question of the germination of spermatia takes on new meaning. If spermatia placed on an infection, but not in actual contact with surface hyphae of the rust, can perform the limited growth necessary to make that contact, fusion and diploidization are made possible.

This has led to a renewed search for germinating spermatia. Hanna (*35*) saw germinating spermatia of *Puccinia graminis*, one spermatium attaining a length of 15μ, and believed that they grew in through the spermogonia and on to the aecia. In *P. graminis* (*2*, *pl. 11*, *A*, *E*) and *P. coronata* (*4*, *pl. 11*, *F*), germinating spermatia were found at or near spermogonia and apparently growing toward the ostiole. The absence of fusions in aecia of these species makes it probable that germinating spermatia, while growing into spermogonia, fuse with the native hyphae there at once. In flax rust (*5*, *8*) germinated spermatia have been found, and there is indirect evidence

that spermatia may make a considerable growth, for, contrary to what was found in *Puccinia*, fusions in young aecia of paired cells to form the basal cells have been found by hundreds (*5*, *8*, *33*). This is presumptive evidence that the mycelium of spermatial origin maintains an independent existence during the 24 hours between spermatization and the fusions in the young aecia. In *P. sorghi*, Rice (*44*) found an occasional germinating spermatium, and in the present investigation an abundance of spermatia in all stages of germination have been found, in which the germ tubes grew into the host plant.

From the point or points of entry of spermatial nuclei into haploid hyphae, in *Puccinia* at least, spermatial nuclei and their progeny spread rapidly through the mycelium.

It might be supposed that in the closely septate mycelium of a rust only substances in solution could pass through a septum from one cell of a hypha to the next. There is increasing evidence, however, that a septum is no barrier to either nucleus or cytoplasm. In both uredial and aecial generations of rusts it frequently has been noted that the older and less active parts of the mycelium contain little or no stainable content and that the cytoplasm of the whole mycelium has been concentrated in the growing tips of the vegetative hyphae and in the reproductive areas. It was earlier assumed (*1*, *p. 1272*) that this efficient conservation of living material was achieved by a breaking down of the cytoplasm into soluble compounds that could pass readily through the septa. In view of the fact, however, that nuclei have been found passing through septa in a number of rusts (*10*, *11*, *12*, *44*, *47*, and in the present work), it seems probable that the cytoplasm, which is composed of particles much smaller than a nucleus, can also pass through.

Andrus (*10*) states that in *Uromyces* the spermatial nuclei enter stomatal hyphae and pass directly to the aecia without diploidizing the mycelial hyphae traversed. In corn rust, on the contrary, 24 hours after the entrance of spermatial nuclei 60 percent of the mycelial cells from one end of the mycelium to the other contain more than one nucleus. It must be assumed that a diploidization process is at work here. The initial binucleate cells at the point or points of entrance could, to be sure, have made some sporophytic growth during that time, but they could hardly have produced so much and such widespread sporophytic mycelium as to exceed in amount the earlier haploid growth. Only a system of diploidization of the already existing haploid mycelium by means of successive nuclear divisions and migrations of the introduced nuclei, such as has been described for other basidiomycetes (*15*, *16*, *38*), can explain the results.

The advantage of mycelial diploidization is obvious. If, as Andrus (*10*) finds in *Uromyces*, spermatial nuclei pass direct to the aecium without diploidizing the mycelium passed through, one spermatization would fertilize only the aecia then ready, and repeated spermatizations would be needed for all aecia formed later. But with widespread mycelial diploidization following the first spermatization, as in *Puccinia*, all subsequent growth of the mycelium will be provided with nuclei of both sexes, and all aecia initiated thereafter assured of normal development. One fertilization suffices for the lifetime of the infection.

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In higher plants the male and female nuclei fuse as soon as they meet. This makes for rigidity and uniformity in the life cycle with small chance of variation in the sequence of nuclear events. In the higher fungi (ascomycetes, rusts, smuts, and Agaricales, on the contrary, the male and female nuclei remain distinct although dwelling together until the end of the sporophyte generation. By the device of conjugate divisions the male and female complement of chromatin may be passed on in regular fashion to each new sporophyte cell in turn throughout the sporophyte generation. Not infrequently, however, the arrangement is less regular and exact than this and in some species the cells are typically multinucleate. Moreover, nuclei wander from one part of a mycelium to another and, when anastomoses form between two mycelia, nuclei move from one mycelium to another. This necessitates a modification of the definition of "gametophyte" and "sporophyte" as applied to fungi, for a gametophytic cell may or may not contain n chromosomes and a sporophytic cell may or may not contain $2n$ chromosomes. Apparently, the male and female haploid nuclei are the essential units to be considered, and what makes a mycelium gametophytic is that it contains nuclei of only one sex while a sporophytic mycelium contains nuclei of both sexes.

Because male and female nuclei remain separate in fungi and can move about independently of each other, there is much greater flexibility in the time, place, and mode of shifting from sporophyte to gametophyte and from gametophyte to sporophyte than would be possible in higher plants.

The following are some of the ways in which the shift from the gametophyte to the sporophyte can be achieved: (1) By the interchange of nuclei between two overlapping and anastomosed haploid mycelia of opposite sex (*16*, *17*, *30*); (2) by the contribution from a diploid mycelium through connecting anastomoses to a haploid mycelium of nuclei of the sex that it lacks (*15*, *16*, *17*, *29*); and (3) by the formation of oidia (Agaricales), microconidia (ascomycetes), or spermatia (rusts), on both the male and the female haploid mycelia. These, if carried by insects or other agents to mycelia of the opposite sex, fuse with them either directly or after germination (*2*, *3*, *4*, *5*, *6*, *7*, *8*, *9*, *10*, *13*, *14*, *23*, *24*, *25*, *26*, *28*, *30*, *31*, *32*, *42*, *44*).

In many fungi any exposed haploid hypha can function as a receptive hypha for nuclei of microspores of the opposite sex; in others only the special receptive organs (*31*, *32*) can so serve.

In an agaric (*14*) and an ascomycete (*28*) the mycelia grown from male and female microspores can diploidize each other and lead to the development of the normal fruiting body. No corresponding case is known in rusts. Spermatia can germinate but, so far as yet observed, male and female spermatial hyphae cannot anastomose with each other and give rise to aecia.

In some heterothallic ascomycetes (*20*, *31*, *32*, *34*) both male and female mycelia produce the coiled hyphae hitherto regarded as female organs, but neither the male nor the female can give rise to the normal fruiting body without the accession of nuclei of the opposite sex.

All these phenomena, so puzzling to interpret if one insists on regarding the microspores (oidia, microconidia, spermatia) as male cells and the hyphae that serve to receive them (whether just ordinary haploid

hyphae or organs specially differentiated for this function) as female, become intelligible if a mycelium is regarded as gametophytic if it contains only male or only female nuclei and is considered sporophytic if it contains both male and female nuclei. Any device, "regular" or irregular, that suffices to bring together male and female nuclei into the same mycelium is "fertilization", and this fertilization is in many cases followed by a diploidization process that renders the whole mycelium bisexual or sporophytic. As Andrus (*10, p. 556*) has well said:

> They [the sperm and egg mechanisms] may be no more male and female than any two other organs of the individual. In many of these species they are a means by which alone diploidization is possible.

There is much less variability in the time, place, and method of shifting from the sporophyte to the gametophyte in the higher fungi than in the shifting from gametophyte to sporophyte. At the end of the sporophyte generation, in the basidium, the ascus, the teliospore, or the chlamydospore, the male and female nuclei fuse and, either at once or after a resting period, undergo the reduction divisions that restore the haploid condition. Other means are known, however, of achieving this transition. In *Pholiota aurivella* Batsch. (*46*) the diploid mycelia produce oidia of several types, in one of which uninucleate haploid oidia are formed that give rise to ordinary haploid mycelium.

Not only can two haploid mycelia diploidize each other or a diploid mycelium contribute the needed nuclei to a haploid mycelium, but there is at least one case, of an ascomycete (*29*), in which two bisexual mycelia mated and formed hybrids.

In *Botrytis cinerea* Pers. (*36*) both the mycelial cells and the conidia are multinucleate. Two strains differing in vegetative character continued constant through several generations. Grown in juxtaposition, they anastomosed freely and an interchange of nuclei took place. The resulting heterocaryotic form differed in appearance from the two that it was derived from. On being carried through several generations it gave rise to both of the original forms (presumably by accidentally including in a conidium only 1 of the 2 kinds of nuclei) and to intermediate forms (according to the percentage of the 2 kinds present). Cases such as this may go far to explain the sectorial "mutations" of imperfect fungi, and illustrate again the great range of possibilities when different nuclei dwell together for long periods without fusing.

SUMMARY

Puccinia sorghi Schw. is a full-cycle heteroecious, heterothallic rust producing spermogonia and aecia on oxalis and uredia and telia on corn.

The sporidium enters through an epidermal cell of the oxalis leaf and grows into haploid mycelium of uninucleate cells. This mycelium produces numerous spermogonia at both the upper and lower surface of the leaf. Very small haploid aecia form in abundance near the lower epidermis. Stomatal hyphae are produced, chiefly adjoining spermogonia and aecia.

In the absence of fertilization the mycelium makes good growth but produces no fertile aecia. Spermogonia remain active for a considerable period, producing spermatia and replacing old paraphyses by fresh ones. New spermogonia form in the fresh marginal growth

of the infection. The small haploid aecia are short-lived but new ones keep forming in the margin as the mycelium spreads. Stomatal hyphae die quickly, but new ones push into stomatal apertures alongside the dead ones and other stomata adjoining marginal growth become occupied.

Only when the two sexes meet do fertile aecia develop. This may be brought about by anastomoses between two mycelia within the leaf or by the transfer of spermatia of one sex to the surface of an infection of opposite sex. "Spermatization" can happen in several ways under natural conditions. Crowded infections can become confluent and their spermatia mixed. If several separate infections occur on one leaf the photeolic motions of the oxalis leaflets can bring the surface of two infections into contact and result in an interchange of spermatia. In more scattered infections spermatia are interchanged by visiting insects or other agents.

Fertilization can take place in infections 10 days old or at any time thereafter as long as the mycelium lives, and is equally effective whether the spermatia are placed on the upper or the lower surface of the infection.

The entrance of spermatial nuclei into haploid hyphae takes place in several ways. A spermatium placed on a spermogonium becomes attached to a paraphysis and its nucleus passes over into the paraphysis and moves in through it to the mycelium within the leaf. A spermatium placed in contact with a stomatal hypha contributes its nucleus to this hypha. Under favorable conditions a spermatium not in contact with any surface hypha can germinate and grow into a slender hypha, which enters the nearest stoma and so becomes effective.

Twenty-four hours after spermatial nuclei enter a mycelium, 60 percent of the mycelial cells contain more than one nucleus. This rapid diploidization of vegetative mycelium is achieved, in the main, by rapid divisions and migrations of the introduced nuclei. Haploid aecia at the right stage are also quickly diploidized and progress at once to spore formation. Six days after spermatization the first aecia open and free spores. One fertilization suffices for a lifetime. All aecia forming after fertilization mature normally.

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STUDIES ON THE POSSIBLE ORIGIN OF PHYSIOLOGIC FORMS OF SPHACELOTHECA SORGHI AND S. CRUENTA[1]

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INTRODUCTION

Two species of the kernel smuts of sorghum (*Sorghum vulgare* Pers.) caused by *Sphacelotheca sorghi* (Link) Clinton and *S. cruenta* (Kühn) Potter are known to consist of pathogenically and otherwise distinct physiologic forms. Three forms of *S. sorghi* were originally identified by Tisdale, Melchers, and Clemmer (*27*)[3] and designated by them as the common, milo, and feterita strains. Later they were referred to as forms 1, 2, and 3, respectively (*21*). Two additional forms, 4 and 5, were identified by Melchers, Ficke, and Johnston (*22*), who reported also other characteristics whereby physiologic forms of *S. sorghi* may be distinguished, namely, length of sori in the smutted panicle, color of membranes surrounding the sori, and the degree of rupturing of the membranes. More recently Melchers (*20*) has identified two pathogenically distinct strains of *S. cruenta*.

The exact origin of these forms has never been demonstrated, although variations found in both the macroscopic and microscopic appearance, as well as in pathogenicity, would strongly suggest that new forms of these organisms may arise as a result of hybridization. For example, the original collection of *Sphacelotheca sorghi* on milo was found by Tisdale et al. (*27*) to have certain characteristics somewhat resembling those of *S. cruenta*. The membranes enveloping the sori seemed in some cases to be intermediate in type and to contain two types of sterile cells, (1) the elongated type of small diameter, adhering in chains, characteristic of *S. sorghi*, and (2) the larger, spherical type, adhering rather loosely in clumps, characteristic of *S. cruenta*. Further indication that new forms of these smuts may arise by hybridization has recently been reported by the writer (*24*). Both *S. sorghi* and *S. cruenta* were found to be heterothallic. Monosporidial lines failed to produce smut in the host plant, but when plants were inoculated with two monosporidial lines of opposite sex, whether from the same species or from two different species, smutted heads were produced.

Theoretically, the smut produced as a result of these intraspecific and interspecific crosses should contain factors governing the general morphology of the smutted panicles, which, in segregation and recombination, might be expected to produce intergrading forms of the two species of smut similar to those observed by Tisdale et

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[3] Reference is made by number (italic) to Literature Cited, p. 1085.

al. (*27*). Furthermore, what is more important, these crosses should contain factors for pathogenicity from both parents, and a recombination of these factors should develop new strains that may differ in virulence from either of the parents. In this study of the origin of physiologic forms of *Sphacelotheca sorghi* and *S. cruenta*, the writer therefore investigated (1) the sexuality of the two organisms with reference to the capacity of various combinations of monosporidial lines to cause infection in the host and (2) the progeny from intraspecific and interspecific crosses with reference to the general morphology of the smutted panicles and the pathogenicity of lines from hybrid chlamydospores.

MATERIALS AND METHODS

Single sporidial lines used in these tests were isolated from promycelia of chlamydospores of forms 1, 2, and 3 of *Sphacelotheca sorghi*, form 1 of *S. cruenta*, and from F_1 intraspecific and interspecific hybrids. The method used in isolating single sporidia was similar to the one described by Dickinson (*9*) and Hanna (*14*) except that the isolating needle was attached to a Chambers micromanipulator. Single chlamydospores were placed on hanging drops of a 2-percent potato-dextrose agar, and when they had germinated the sporidia were removed from the promycelia and propagated separately. Subcultures of these lines, to be used as inoculum, were prepared by growing each line separately in a 4-percent carrot extract. The cultures were allowed to develop 3 weeks before being used to inoculate sorghum plants.

Reed kafir sorghum (C. I. 628),[4] which is susceptible to all known collections of *Sphacelotheca sorghi* and *S. cruenta*, was used in all the experiments on sex and also in the study of the progeny of segregating hybrid smuts. When the plants were about 5 weeks old they were inoculated with the sporidial suspensions by means of a hypodermic syringe. When a combination of two monosporidial lines was used the two cultures were mixed just before inoculation. Ten plants were inoculated for each test. The plants were grown singly in pots in the greenhouse and 6 days after inoculation were transplanted to the field. In some of the tests the plants were allowed to mature in the greenhouse.

The physiologic forms of *Sphacelotheca sorghi* are referred to in this paper as forms S1, S2, and S3, and a form of *S. cruenta* as C1. Where different chlamydospores of a particular form or collection were used they are distinguished by a roman numeral. A record was kept of the position on the promycelium occupied by each sporidium, and for convenience the sporidia are numbered 1, 2, 3, and 4 from the tip of the promycelium to the chlamydospore. Thus S1–II–4 would refer to a sporidium isolated from the fourth segment from the tip of the promycelium, from chlamydospore II of form 1 of *S. sorghi*. In the lines that are of hybrid origin, the letter H is substituted for the form identification letter and number.

Heidenhain's iron-alum haematoxylin was used as a nuclear stain. The procedure was the same as that outlined by Holton (*17*) for staining nuclei of the oat-smut fungi except that for a bleaching agent diaphanol was substituted for the hydrogen peroxide.

[4] C. I. denotes accession number of Division of Cereal Crops and Diseases.

SEXUALITY

SPORE GERMINATION AND CYTOLOGY

A number of investigators have studied the germination of *Sphacelotheca sorghi* (*5, 6, 8, 19, 23*) and *S. cruenta* (*4, 6, 19*). However, as information on the nuclear behavior is also prerequisite to an understanding of sexuality, observations were made on the cytology of these two species.

The nuclear behavior in germinating spores of *Sphacelotheca sorghi* and *S. cruenta* is essentially the same, and it is apparently similar to that in *Ustilago zeae* (Beckm.) Ung. as described by Hanna (*15*) and Christensen (*7*). The mature chlamydospore contains a single diploid nucleus, which starts to divide as the promycelium develops. As the promycelium elongates, one of the daughter nuclei migrates into it, while the other remains in the chlamydospore. Both daughter nuclei then divide, and in the mature promycelium there may be found either 3 or 4 nuclei. When 3 nuclei are found in the mature promycelium, a fourth may be seen in the chlamydospore. From 1 to 3 septa are laid down. In most cases the first-formed septum separates the nuclei of the first division, while the other 2 separate the remaining daughter nuclei of the second division. When 3 cross walls are formed, the sporidia seem to develop at definite positions on the promycelium, namely, at the tip and at each remaining segment. Under these conditions the daughter nuclei divide, and before the sporidium matures 1 of the nuclei migrates into the young sporidium, while the other remains in the promycelial cell. Successive generations of sporidia are then produced in the same manner.

Considerable irregularity was found in the number of septa formed and the position of the primary sporidia on the promycelium. It is not uncommon to find a single cross wall in the promycelium with 3 or 4 primary sporidia budding from the upper segment and several more developing simultaneously at the junction of the promycelium and the chlamydospore. Furthermore, some of the promycelia produce sporidia and, in addition, peglike structures, which in many cases develop into hyphal threads. A single primary sporidium may be produced at any one segment while the 2 or 3 other segments produce hyphal tubes. Irregularities in sporidial production were most common on promycelia of F_1 chlamydospores of some of the interspecific crosses. Of 109 germinated F_1 chlamydospores from an S1–I–2 × C1–I–2 cross only 8 formed promycelia having 4 distinct segments and a primary sporidium at each segment. The promycelia of the remaining 101 chlamydospores produced both sporidia and the peglike structures. The sporidia produced from these hybrids were normal, as evidenced by the fact that when paired with sexually compatible lines they produced smut in the host plant. However, the majority of the peglike structures failed to develop into the hyphal tubes, possibly largely as a consequence of genetic sterility.

SEXUAL COMPATIBILITY

The inheritance of factors governing the sex of sporidia and the capacity of various combinations of monosporidial lines to cause infection in the host has been studied in a number of the cereal-smut fungi. Evidence of 2 sexual groups in *Ustilago levis* (Kell. and Sw.)

Magn. and *U. hordei* (Pers.) Kell. and Sw. has been reported by Dickinson (*11*), while Hanna and Popp (*16*) and Holton (*17*) found 2 in *U. levis* and *U. avenae* (Pers.) Jens. Hanna (*15*) found that the 4 sporidia on certain promycelia of *U. zeae* fell into 2 sexual groups and those on others belonged to 4. Christensen (*26*) later reported evidence of at least 24 sexual groups in *U. zeae*. Flor (*13*) reported evidence of a number of sexual groups in both *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. The sexual behavior of monosporidial lines from 3 promycelia of *Sphacelotheca sorghi* and 3 of *S. cruenta* has recently been reported by the writer (*24*). Two sexual groups were identified in each species, and there was evidence of segregation of factors for sex in the first, second, and possibly a third division of the fusion nucleus. In the present investigation the sexual behavior of lines from 10 promycelia of *S. sorghi* and 10 of *S. cruenta* was studied, and the data are presented in table 1. The results obtained corroborate those of the earlier investigations.

TABLE 1.—*Sexual groupings of monosporidial lines isolated from 10 chlamydospores of Sphacelotheca sorghi and from 10 of S. cruenta*

S. SORGHI

Sporidium no.	Chlamydospore no. and sexual group of sporidium [1]									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1	A	A	A	A	A	A	A	A	A	A
2	B	B	B	B	A	B	B	A	B	B
3	A	B	B	B	B	B	A	A	B	B
4	B	B	A	B	B	B	B	B	B	B

S. CRUENTA

1	A	A	A	A	A	A	A	A	A	A
2	B	A	B	B	B	A	B	A	B	A
3	B	A	B	A	B	B	B	B	B	B
4	A	B	B	B	A	B	A	B	B	B

[1] The sexual groups into which the monosporidial lines fall are indicated by A and B. The groups for each chlamydospore were determined separately so that the A or B group of one chlamydospore is not necessarily identical with the A or B group of another chlamydospore.

Christensen (*7*) reported that in *Ustilago zeae*, when the monosporidial lines isolated from a promycelium are paired in all possible combinations, they may fall in 1 to 4 sexual groups, and that when paired with sporidia from other promycelia they frequently belong to many distinct sexual groups. In the present investigations all possible paired combinations were made between monosporidial lines of the same segment number isolated from the promycelia of the 10 chlamydospores of *Sphacelotheca sorghi* referred to in table 1. The data obtained are presented in figure 1. From the ability shown by paired lines to produce smut in the host plant, it is evident that the sporidia from the same segment number of each of the 10 promycelia represent more than one sexual or compatibility group. It is probable that even a larger number of sexual groups may have been identified within these lines if the apparently incompatible lines had been mated with lines from other chlamydospores. The fact that smut is not produced in the host plant as a result of inoculation

Segment no. 1

	I	II	IV	VII	III	V	VI	VIII	IX	X	Compatibility group
I	−	−	−	−	−	+	+	−	−	+	1
II	−	−	−	−	−	+	+	+	+	+	2
IV	−	−	−	−	−	+	+	+	+	+	2
VII	−	−	−	−	−	+	+	+	+	+	2
III	−	−	−	−	−	−	−	+	−	+	3
V	+	+	+	+	−	−	−	−	−	−	4
VI	+	+	+	+	−	−	−	−	−	−	4
VIII	−	+	+	+	+	−	−	−	−	−	5
IX	−	+	+	+	−	−	−	−	−	−	6
X	+	+	+	+	+	−	−	−	−	−	7

Segment no. 2

	I	II	III	IV	V	VII	VIII	VI	X	IX	Compatibility group
I	−	−	−	−	−	−	−	+	+	−	1
II	−	−	−	−	−	−	−	+	+	−	1
III	−	−	−	−	−	−	−	+	+	+	2
IV	−	−	−	−	−	−	−	+	+	+	2
V	−	−	−	−	−	−	−	+	+	+	2
VII	−	−	−	−	−	−	−	+	+	+	2
VIII	−	−	−	−	−	−	−	+	+	+	2
VI	+	+	+	+	+	+	+	−	−	−	3
X	+	+	+	+	+	+	+	−	−	−	3
IX	−	−	+	+	+	+	+	−	−	−	4

Segment no. 3

	I	V	VI	IX	X	II	VIII	III	IV	VII	Compatibility group
I	−	−	−	−	−	+	+	+	+	−	1
V	−	−	−	−	−	+	+	+	+	−	1
VI	−	−	−	−	−	+	+	+	+	−	1
IX	−	−	−	−	−	+	+	+	+	−	1
X	−	−	−	−	−	+	+	+	+	−	1
II	+	+	+	+	+	−	−	−	−	+	2
VIII	+	+	+	+	+	−	−	−	−	+	2
III	+	+	+	+	+	−	−	−	−	+	2
IV	+	+	+	+	+	−	−	−	−	−	3
VII	−	−	−	−	−	+	+	+	−	−	4

Segment no. 4

	I	II	III	VI	IV	V	VII	VIII	IX	X	Compatibility group
I	−	−	+	+	−	−	−	+	+	+	1
II	−	−	+	+	−	+	−	+	−	−	2
III	+	+	−	−	+	−	−	−	−	−	3
VI	+	+	−	−	+	−	−	−	−	−	3
IV	−	−	+	+	−	+	−	+	+	+	4
V	−	+	−	−	+	−	−	−	−	−	5
VII	−	−	−	−	−	−	−	+	+	−	6
VIII	+	+	−	−	+	−	+	−	−	−	7
IX	+	−	−	−	+	−	+	−	−	−	8
X	+	−	−	−	+	−	−	−	−	−	9

FIGURE 1.—Results of all possible pairings of 10 monosporidial lines of *Sphacelotheca sorghi* isolated from the same segment number of each of 10 promycelia.

with a given mating does not necessarily mean that the lines are of the same sex, for in some matings incompatibility may be due to the presence of sterility factors. For example, data given by Christensen (*7, table 8*) show the complete intersterility of 63 lines of *U. zeae*. When, however, these lines were mated in other crosses many lines were found to be compatible, thus indicating that segregation of factors affecting pairing had taken place. The incompatibility of some of the 63 lines might then be explained by the presence of sterility factors, which, if taken into consideration as they are in the higher plants, are obviously a disturbing element in determining the number of sexual groups present. Although the data in figure 1 indicate that there are a number of compatibility groups within the lines of the same segment number, the results may be explained by the presence of sporidia of two different sexes. On the basis of compatible matings only, the lines from segment no. 1 of chlamydospores I, II, III, IV, and VII are of one sex, while those from V, VI, VIII, IX, and X are of another; likewise, lines from segment no. 2 of chlamydospores VI, IX, and X represent one sex and those from chlamydospores I, II, III, IV, V, VII, and VIII another sex.

SPORIDIAL FUSION

Sporidial and also hyphal fusions are known to occur in several of the smut fungi (*18*). In the cereal smuts Dickinson (*10*) observed the fusion between *Ustilago hordei* and *U. levis*, and Sleumer (*25*) recently reported sporidial fusions in *U. zeae*. Holton (*17, p. 13*) studied in detail sporidial fusions in *U. avenae* and *U. levis* and summarized the process as follows:

> One sporidium, designated as the active one, assumes a definite active attitude which is evidenced by the production of a short projection that grows in the direction of its nearest sexually opposite sporidium, which appears to be passive. When the projection comes into contact with the passive sporidium, fusion takes place and it is immediately followed by the invariable increase in length of the fusion tube.

In the present study, fusions have been observed between paired lines of sporidia of *Sphacelotheca sorghi*, of *S. cruenta*, between sporidia of different pathogenically distinct forms of *S. sorghi*, and between paired lines of sporidia of *S. sorghi* and *S. cruenta*. The process of fusion was observed to be essentially the same as that reported by Holton (*17*) for *Ustilago levis* and *U. avenae*. Camera-lucida drawings of fused sporidia and hyphal threads are shown in figure 2. The sporidia of *S. sorghi* and *S. cruenta* are predominantly uninucleate. Shortly after fusion an infection hypha develops into which the contents of the paired sporidia pass. As the hypha elongates the contents of the sporidia migrate toward the tip, and associated there may be seen the two nuclei which migrated from the paired sporidia. These dikaryotic hyphae, which presumably constitute the parasitic stage of the fungus, are as a rule aerial in habit, and within 10 hours after the sporidia fuse the hyphae may be seen protruding abundantly from colonies of sexually compatible paired lines. Within 15 hours these colonies are completely covered with white aerial hyphae, whereas only an occasional aerial hypha may be seen in colonies of paired sexually incompatible lines and monosporidial lines.

Bauch (*1, 2, 3*) found that the type of growth of colonies of sexually compatible paired lines of *Ustilago violacea* (Pers.) Fuckel, *Sphace-*

lotheca schweinfurthiana (Thüm.) Sacc., *U. scorzonerae* (Alb. and Schw.) Schroet., and *U. zeae* was distinctly different from that of monosporidial lines or sexually incompatible paired lines, and he considered this a reliable criterion for making sex determinations. Holton (*17*) reported the so-called "Bauch test" to be unreliable for determining the compatibility of lines of *U. levis* and *U. avenae*.

Experiments were made to determine whether this test could be used in the determination of sexual compatibility of lines of *Sphacelotheca sorghi* and *S. cruenta*. All possible pairings were made between lines of each set of 4 sporidia originating from each of the 10 chlamydospores of *S. sorghi* and of *S. cruenta* referred to in table 1, between monosporidial lines of corresponding segment numbers of the 10 chlamydospores referred to in figure 1, between lines of pathogenically distinct forms of *S. sorghi*, and between lines of *S. sorghi* and *S. cruenta*. The type of growth in colonies resulting from these pairings and in the monosporidial lines was then studied on 2-percent malt agar.

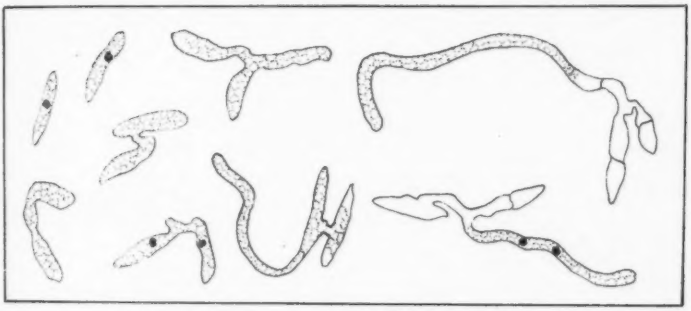


FIGURE 2.—Camera-lucida drawings of fused sporidia of *Sphacelotheca sorghi*, line S1–I–2, and *S. cruenta* line C1–I–2. × 600.

Figure 3 is from a photograph that shows differences in growth types. The results obtained from all of these pairings indicate that the Bauch test is a reliable criterion of compatibility between intraspecific and interspecific lines of *S. sorghi* and *S. cruenta*. The sporidial mass of all colonies was examined microscopically, and fused sporidia were found only in those colonies that produced the abundant growth of aerial hyphae. Furthermore, only those cultures in which fused sporidia were present caused the flecking reaction, referred to by the writer in a previous paper (*24*), and the subsequent production of chlamydospores in a susceptible sorghum plant.

HYBRIDIZATION AND SEGREGATION

The production of hybrid chlamydospores as a result of intraspecific and interspecific crossing has been reported in several of the cereal-smut fungi. Hanna and Popp (*16*) and Holton (*17*) obtained hybrid chlamydospores in the oat-smut fungi as a result of crosses between physiologic forms of a single species and of crosses between the two species *Ustilago levis* and *U. avenae*. Flor (*13*) obtained hybrid chlamydospores in both intraspecific and interspecific crosses

of *Tilletia levis* and *T. tritici*. Hybridization between *Sphacelotheca sorghi* and *S. cruenta* was recently reported by the writer (*24*). The interspecific crosses produced sori macroscopically characteristic of *S. cruenta*, while the intraspecific crosses produced sori macroscopically characteristic of the species to which each belonged.

In the development of these F_1 hybrids, obviously there is opportunity for a recombination of factors governing the morphology of the sori and the cultural characteristics and pathogenicity of the lines. In order to determine the degree to which these factors may recombine, a study was made of the segregating progeny of an interspecific cross, S1–I–2×C1–I–2. A diagram indicating the method of procedure is given as figure 4. The S1–I–2 parent used in the cross

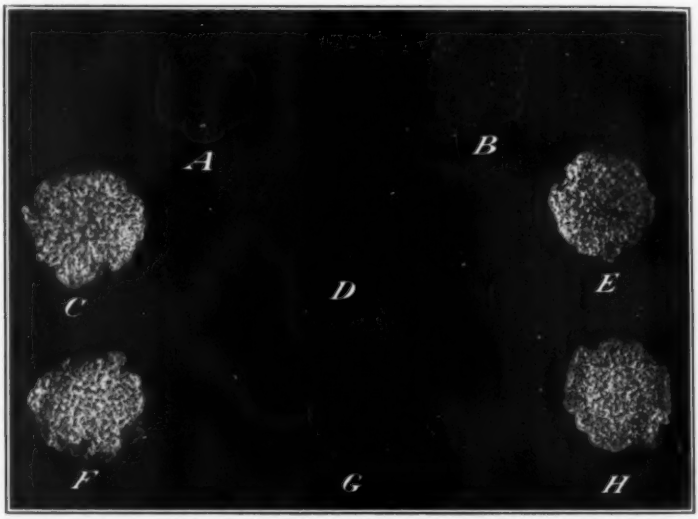


FIGURE 3.—Type of growth produced by monosporidial lines of *Sphacelotheca sorghi* and *S. cruenta* and sexually compatible and incompatible combinations: *A* and *B* are monosporidial lines of *S. sorghi* and *S. cruenta*, respectively; *C*, paired compatible lines of form 1, *S. sorghi*; *D*, paired incompatible lines of *S. sorghi*; *E*, paired compatible lines of form 1 and form 2, *S. sorghi*; *F*, compatible lines of form 1, *S. cruenta*; *G*, incompatible lines of form 1, *S. cruenta*; *H*, compatible lines of form 1, *S. sorghi*, and form 1, *S. cruenta*.

was an isolate from the promycelium of a third generation inbred line of *Sphacelotheca sorghi*. The inbred line caused no stunting of the sorghum plants, the sori were relatively small, and their peridia were reddish brown and not ruptured at maturity. The sterile cells within the sori were of the elongated type. The C1–I–2 parent was an isolate from the promycelium of a third generation inbred line of *S. cruenta*. This inbred line caused obvious stunting of the host plant, the sori were relatively long, and the peridia were gray and were completely ruptured at maturity. The sterile cells within the sori were of the spherical type. The sori of the F_1 progeny resulting from this interspecific cross were of the *S. cruenta* type, indicating the dominance of *S. cruenta* factors in the F_1 hybrid.

Ninety single sporidia were isolated from the promycelia of 37 of the F_1 hybrid chlamydospores isolated from a single sorus. As indicated in table 2, 52 of these monosporidial lines were paired in

a back cross with cultures of the recessive parent S1–I–2, and 38 isolates were paired in a back cross with cultures of S1–I–3, an isolate of opposite sex from the same promycelium of the recessive parent. The results of inoculation with these paired lines are recorded in table 2, and representative types of the progeny recovered are shown in figure 5. In the segregating progeny of this first-generation back cross, phenotypes were recovered resembling macroscopically *Sphacelotheca sorghi*, *S. cruenta*, and a third type which was distinctly an intermediate type, having some of the characteristics common to both species. Both types of sterile cells were found in all three progeny types. The elongated sterile cells of small diameter, which adhere in chains, predominated in sori macroscopically characteristic of *S. sorghi*, while the larger spherical sterile cells, which ad-

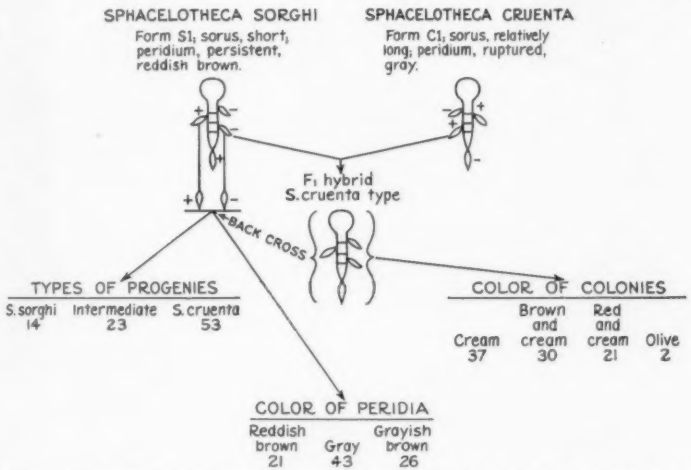


FIGURE 4.—Diagrammatic representation of the procedure followed in a study of the progenies of the interspecific hybrid S1–I–2 × C1–I–2.

here rather loosely in clumps, predominated in the *S. cruenta* types. The distribution of the elongated and spherical cells was more even in the intermediate types, thus adding to the difficulty of classifying the progeny. The two types of sterile cells found in a single sorus of the intermediate type shown in figure 5 are presented in figure 6.

From the data given in table 2 it is evident that there was segregation of the factors which govern the color of peridia of sori. As previously noted, the peridia of the inbred *Sphacelotheca sorghi* parent were reddish brown in color, whereas those of the *S. cruenta* parent were gray. Factors for the gray color were dominant in the F_1 hybrid, and the progeny from the back cross segregated for color into three groups, reddish-brown, grayish-brown, and gray, in the proportion 35:29:26. These distinct differences in the color of peridia are shown in plate 1, *A*, *a*, *b*, and *c*. Factors for color apparently are not linked with those governing species characters of the intermediate and *S. cruenta* types. Of the 53 progenies of the *S. cruenta* type, 12 had

reddish-brown peridia, 19 grayish-brown, and 22 gray. Of the intermediate type, 10 were reddish-brown, 9 grayish-brown, and 4 gray. There is, however, some evidence of linkage between factors governing the *S. sorghi* type of physiologic form 1 and the reddish-brown color, for of the 14 *S. sorghi* type progenies, 13 had reddish-brown peridia and 1 had grayish-brown. The intensity of color of the peridia was

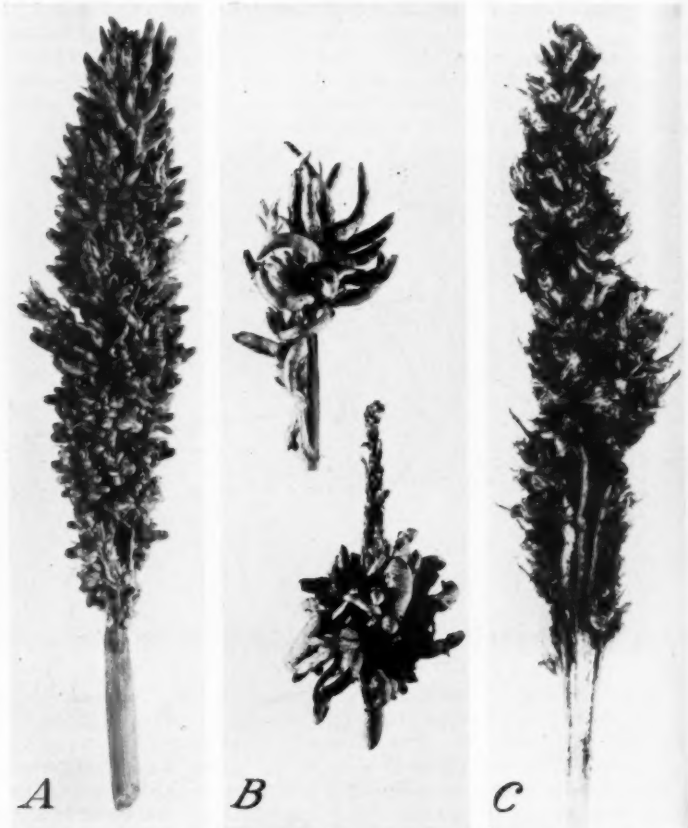
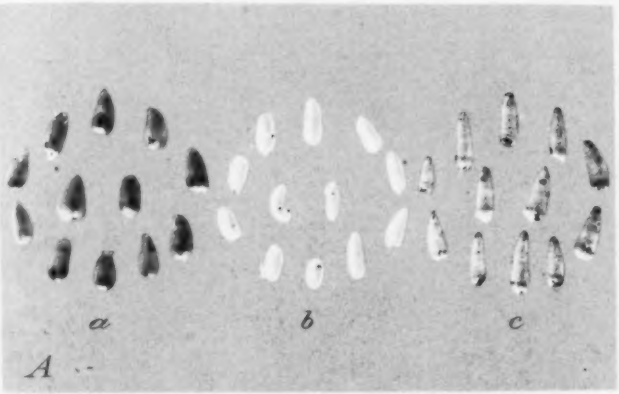
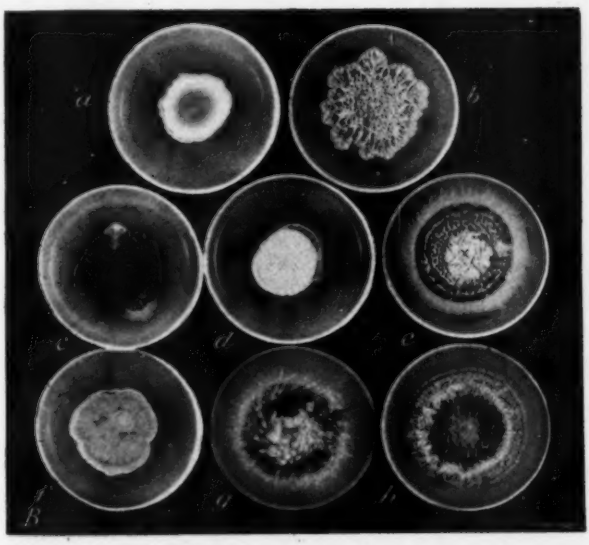


FIGURE 5.—Representative types of segregating F_2 progeny of the interspecific hybrid S1-I-2 × C1-I-2: *A*, *Sphacelotheca sorghi* type; *B*, intermediate type; *C*, *S. cruenta* type.

found to vary somewhat, depending on the environment in which the plants were grown. Observations on color recorded in table 2 were made on plants growing in the field. The stalks of representative plants were cut back and the secondary shoots of transplants were allowed to develop in pots in the greenhouse. Under these environmental conditions the color of the peridia of the smutted secondary shoots was somewhat less intense than that observed in plants grown in the field.





A, Sori in segregating progenies of the interspecific cross 81-I-2 × C1-I-2, showing differences in color of peridia: *a*, Reddish-brown segregate; *b*, gray segregate; *c*, grayish-brown segregate. *B*, Colonies, of monosporidial origin, of *Sphacelotheca sorghi*, line 81-I-2, and *S. cruenta*, line C1-I-2, and of the F_1 progenies of the interspecific hybrid: *a*, Parent line 81-I-2; *b*, parent line C1-I-2; *c–h*, F_1 progenies of the interspecific hybrid.

TABLE 2.—*Data on segregation in F_2 progenies of the interspecific hybrid S1–I–2 × C1–I–2*

Hybrid chlamydospore no.	Sporidium no.	Back-cross parent S1–I–2	Back-cross parent S1–I–3	Species which progeny resembled	Color of peridium	Degree of stunting[1]
I	1		×	Intermediate	Grayish brown	0
	2	×		do	do	0
	3	×		do	do	0
II	1		×	*S. sorghi*	Reddish brown	0
	2		×	do	do	0
	3	×		*S. cruenta*	Gray	+
	4		×	*S. sorghi*	Reddish brown	0
III	1	×		Intermediate	do	0
VI	4		×	*S. cruenta*	Grayish brown	+
VII	3	×		*S. sorghi*	Reddish brown	0
	4	×		do	Grayish brown	0
VIII	1	×		*S. cruenta*	Gray	0
	3	×		*S. sorghi*	Reddish brown	0
	4		×	Intermediate	do	0
IX	2	×		*S. cruenta*	do	+
	3		×	do	Grayish brown	++
X	1		×	Intermediate	Reddish brown	0
	4	×		*S. cruenta*	Grayish brown	0
XI	2	×		do	Gray	++
	3	×		do	do	+
XII	1		×	*S. sorghi*	Reddish brown	+
	2	×		*S. cruenta*	Gray	+
	3	×		do	Grayish brown	+
	4	×		do	do	0
XIII	1		×	Intermediate	do	+
	2	×		*S. cruenta*	do	+
	3		×	do	Gray	+
	4	×		Intermediate	do	+
XIV	1		×	*S. cruenta*	Grayish brown	++
	2	×		do	do	++
XV	2	×		*S. sorghi*	Reddish brown	0
	4		×	Intermediate	Grayish brown	0
XVI	1		×	*S. cruenta*	do	0
	4	×		do	Gray	+
XVII	2	×		do	do	0
	3	×		do	Grayish brown	0
	4		×	*S. sorghi*	Reddish brown	0
XVIII	2		×	*S. cruenta*	Grayish brown	0
	3	×		do	Reddish brown	+
XIX	1	×		do	Gray	+
	2	×		do	Grayish brown	+
	3	×		Intermediate	do	+
	4		×	do	Reddish brown	+
XX	1	×		*S. cruenta*	Grayish brown	0
	2		×	do	do	0
	3		×	Intermediate	do	0
	4	×		*S. cruenta*	do	+
XXI	1		×	Intermediate	do	0
	3		×	do	Gray	0
	4		×	*S. cruenta*	do	0
XXII	2	×		do	Reddish brown	+
	3		×	do	Gray	+
	4	×		do	Grayish brown	0
XXIII	2	×		do	do	+
	3	×		do	do	+
XXIV	1	×		Intermediate	do	0
	2		×	do	Reddish brown	0
	3		×	do	Grayish brown	0
XXV	4	×		*S. sorghi*	do	+
XXVI	1	×		do	Reddish brown	0
XXVII	1	×		*S. cruenta*	Gray	0
	3	×		*S. sorghi*	Grayish brown	0
XXVIII	3	×		*S. cruenta*	Gray	+
	4	×		do	Grayish brown	+
XXIX	2	×		Intermediate	Reddish brown	+
	3	×		*S. cruenta*	Gray	0
XXX	1		×	Intermediate	Grayish brown	0
	3		×	*S. cruenta*	do	0
XXXI	1		×	do	Gray	+
	2	×		do	Grayish brown	+
XXXII	2		×	do	do	++
	4		×	do	Gray	+
XXXIII	1	×		do	Grayish brown	0
	2	×		Intermediate	Gray	0
	3	×		do	do	0
XXXIV	1		×	*S. sorghi*	Reddish brown	+
	4	×		*S. cruenta*	Gray	+

[1] 0 denotes no stunting; +, plants stunted 6–18 inches; ++, plants stunted to about one-third their normal height.

TABLE 2.—*Data on segregation in F_2 progenies of the interspecific hybrid S1–I–2 × C1–I–2*—Continued

Hybrid chlamydospore no.	Sporidium no.	Back-cross parent		Species which progeny resembled	Color of peridium	Degree of stunting
		S1–I–2	S1–I–3			
XXXV	2		×	*S. cruenta*	Gray	++
	4		×	do	do	+
XXXVI	1		×	do	Grayish brown	+
	3	×		do	Gray	0
	4		×	do	Grayish brown	++
XXXVII	2	×		do	do	+
	4	×		do	do	0
XXXVIII	1	×		do	Gray	+
	2	×		do	Reddish brown	+
	3		×	Intermediate	Grayish brown	0
	4		×	do	do	0
XXXIX	1	×		*S. sorghi*	Reddish brown	0
	3		×	*S. cruenta*	Gray	+

It has been generally observed that plants infected with *Sphacelotheca cruenta* are considerably shorter than healthy plants or those

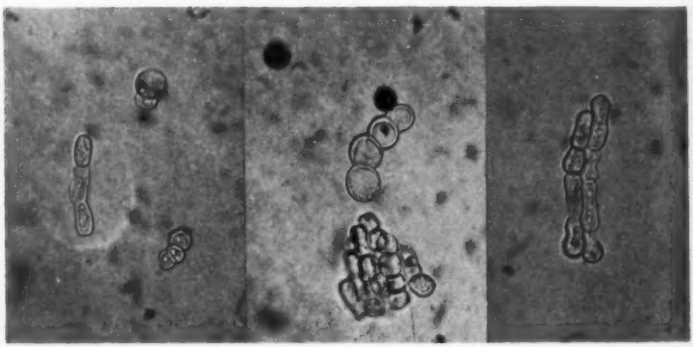


FIGURE 6.—Elongated and spherical types of sterile cells found in a single sorus of the intermediate-type sori shown in figure 5, *B*.

infected with *S. sorghi*. In the present investigation, observations were made on the degree to which plants were stunted when infected with smut of any one of the three progeny types. From the data recorded in table 2 it is evident that segregation took place for factors affecting the degree to which the smut of the *S. cruenta*, *S. sorghi*, or the intermediate types might stunt the host plant. Seven of the 53 *S. cruenta* type progenies caused stunting to the extent that the plants were only about one-third their normal height (++ reaction), 29 stunted the plants 6 to 18 inches (+ reaction), and 17 caused no stunting (0 reaction). Five of the 23 intermediate type progenies and 3 of the *S. sorghi* type progenies were stunted 6 to 18 inches. These results indicate that factors governing the degree to which the smut may stunt the host plant are not linked with those governing other characters.

CULTURAL CHARACTERS

Differences in the cultural characteristics of the pathogenically distinct common, milo, and feterita strains of *Sphacelotheca sorghi* have been reported by Ficke and Johnston (*12*). More recently the

writer (*24*) studied the cultural characteristics of the four primary monosporidial lines isolated from the promycelia of each of three chlamydospores of *S. sorghi* and of *S. cruenta*. The results indicated that, in both species, the factors governing the cultural characteristics of a line segregate independently of those determining the pathogenicity of strains. To determine further the degree to which factors governing the cultural characteristics of these lines may segregate, a study was made of the growth types of the parent lines, S1–I–2 and

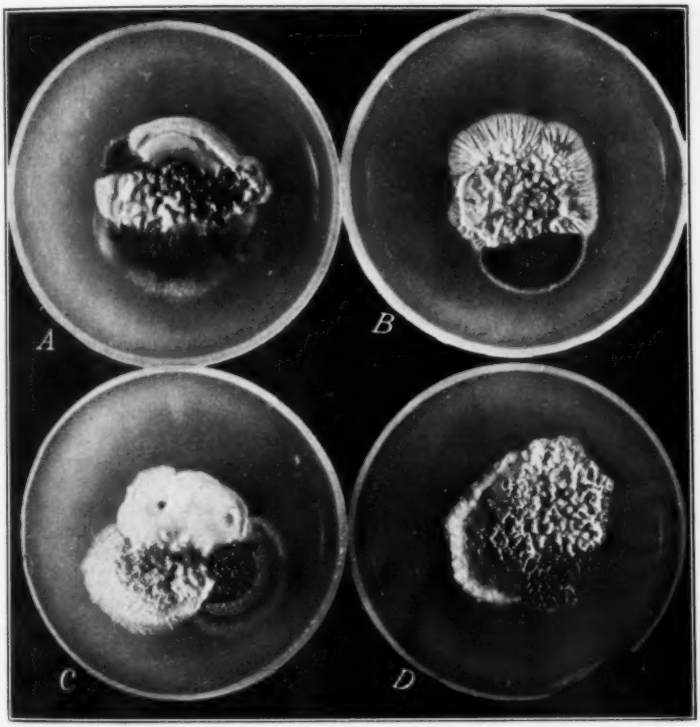


FIGURE 7.—Mutants arising as distinct sectors or patches in cultures of monosporidial lines isolated from promycelia of F_1 hybrid chlamydospores S1–I–2 × C1–I–2. *A*, Line H–IX–2; *B*, line H–XXV–4; *C*, line H–X–4; *D*, line H–XIII–4.

C1–I–2, referred to in figure 4, and of the 90 monosporidial lines isolated as progenies of the F_1 interspecific hybrid. The cultural characteristics of the parent lines and representative segregated progenies are shown in plate 1, *B*.

The cultural characteristics of 2 of the 90 lines, H–XVII–4 and H–XXXIII–2, referred to in table 2, were identical with those of the *Sphacelotheca sorghi* parent line, S1–I–2. However, one of these two lines, H–XXXIII–2, proved to be of opposite sex to the *S. sorghi* parent line, and was evidently heterozygous for factors governing species characters since the smut produced in sorghum plants as a result of inoculations with this line paired in the back cross with the

recessive parent line S1–I–2 was of the intermediate type. The cultural characteristics of one of the other lines, H–XXIX–3, were identical with those of the *S. cruenta* parent. Other lines of the cross were strikingly different from the parent lines in one or more of the following characters: Color of colony, topography, surface, consistency, margin, rate of growth, and tendency to mutate. As regards color of colony, the 90 lines fell into 4 general groups, each of which is represented in natural color in plate 1, *B*. A group of 37 lines was cream in color, as shown in plate 1, *B*, *d*; a second group of 30 lines produced both brown and cream color, as shown in plate 1, *B*, *e* and *g*; a third group of 21 lines produced the cream and red color characteristic of the *S. sorghi* parent line and of the cultures shown in plate 1, *B*, *f* and *h*; the fourth group, containing 2 lines, produced an olive color, as shown in plate 1, *B*, *c*. It was evident from these tests that more than one pair of factors governs the color of colonies. Factors governing any one of the cultural characters mentioned above may segregate independently of the others and of those determining the sex of a line and species characters. For example, in the tests with the 4 primary sporidial lines isolated from the promycelium of chlamydospore H–II, referred to in table 2, it was found that factors for color segregated in the ratio 2 : 1 : 1, for topography 2 : 2, for consistency 4 : 0, for sex 2 : 2, and for type of sorus 3 : 1. In most lines the factors governing cultural characters were very stable; however, in a few of the colonies there was evidence of mutation.

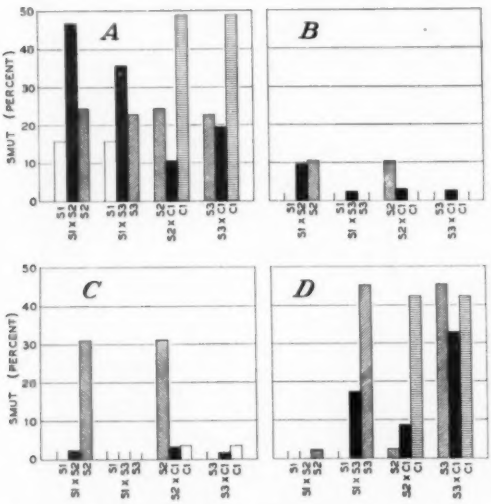


FIGURE 8.—Percentage of infection in sorghums inoculated with physiologic forms of *Sphacelotheca sorghi*, with *S. cruenta*, and with F_1 intraspecific and interspecific hybrid chlamydospores at Dalhart, Tex., in 1931: *A*, Reed kafir; *B*, Fargo straightneck milo; *C*, Dwarf yellow milo; *D*, Kafir × feterita.

When cultured on 2 percent potato-dextrose agar, 4 of the 90 lines isolated from promycelia of the F_1 hybrid chlamydospores mutated frequently. Variants in these 4 lines, H–IX–2, H–X–4, H–XIII–4, and H–XXV–4, appeared in the form of wedge- or fan-shaped sectors or patches, as shown in figure 7. Certain monosporidial isolates from the promycelia of third-generation inbred lines of forms 1, 2, and 3 of *Sphacelotheca sorghi* and form 1 of *S. cruenta* were also stable, while others mutated as frequently as did the monosporidial lines from the hybrid chlamydospores mentioned above. No evidence was obtained of mutation for sex in any of the colonies.

TABLE 3.—*Percentage of smutted plants in sorghums inoculated with physiologic forms of Sphacelotheca sorghi, with S. cruenta, and with F_1 intraspecific and interspecific hybrid chlamydospores at Dalhart, Tex., in 1931*

Variety	Accession no.	Smut form or hybrid	Total plants	Smutted plants	
			Number	*Number*	*Percent*
Reed kafir	C.I. 628	S1	101	16	15.8
		S2	115	28	24.3
		S3	96	22	22.9
		C1	107	52	48.6
		S1×S2	94	44	46.8
		S1×S3	85	30	35.3
		S2×C1	107	11	10.3
		S3×C1	78	15	19.2
Dwarf Yellow milo	C.I. 332	S1	131	0	0
		S2	129	40	31.0
		S3	141	0	0
		C1	150	5	3.3
		S1×S2	131	3	2.3
		S1×S3	139	0	0
		S2×C1	125	4	3.2
		S3×C1	129	2	1.6
Spur feterita	C.I. 623	S1	152	0	0
		S2	146	0	0
		S3	109	0	0
		C1	134	0	0
		S1×S2	122	0	0
		S1×S3	133	0	0
		S2×C1	128	0	0
		S3×C1	97	0	0
Dwarf hegari	C.I. 620	S1	196	0	0
		S2	169	17	10.1
		S3	133	0	0
		C1	156	0	0
		S1×S2	109	3	2.8
		S1×S3	135	0	0
		S2×C1	144	1	0.7
		S3×C1	141	0	0
Kafir × feterita	H.C. 2423 [1]	S1	122	0	0
		S2	96	2	2.1
		S3	111	50	45
		C1	116	49	42.2
		S1×S2	41	0	0
		S1×S3	104	18	17.3
		S2×C1	82	7	8.5
		S3×C1	76	25	32.9
Fargo milo	C.I. 809	S1	96	0	0
		S2	98	10	10.2
		S3	109	0	0
		C1	105	0	0
		S1×S2	114	11	9.6
		S1×S3	95	2	2.1
		S2×C1	100	3	3.0
		S3×C1	117	3	2.6

[1] The letters H.C. indicate the accession number of the Fort Hays Branch Experiment Station of the Kansas State Agricultural Experiment Station.

PATHOGENICITY

It is evident from the results of the foregoing experiments that the intermediate types of the two kernel smuts, differing genotypically and phenotypically from either parent, may be produced under controlled conditions by intraspecific and interspecific hybridization. While the general morphology of the intermediate types are of interest in a study of the origin of forms, the pathogenicity of hybrids as compared with that of parent forms is of equal interest and of greater practical importance. To determine whether or not the factors governing pathogenicity in hybrid lines differ from those in the parent lines, the virulence of forms S1, S2, S3, and C1 was tested in comparison with that of the F_1 hybrids S1×S2, S1×S3, S2×C1, and S3×C1. The results of pathogenicity tests with these lines on six varieties of sorghum are given in table 3, and the results of tests with four of the

varieties are represented graphically in figure 8. Although the data obtained are from tests made in a single season, they indicate definitely that progenies that differ in virulence from either of the parent forms may be obtained by hybridization. On Reed kafir (C.I. 628) the intraspecific crosses were more virulent than were lines of either parent form. Forty-six and eight-tenths percent of the plants became smutted with the hybrid S1×S2, while 15.8 and 24.3 percent became smutted with the parent forms S1 and S2, respectively. Similar differences were recorded for plants inoculated with the hybrid S1×S3 and the parent lines S1 and S3. On this same variety of sorghum, interspecific hybrid lines proved to be less virulent than the parent lines. For example, only 10.3 percent of the plants became smutted when inoculated with chlamydospores of the S2×C1 hybrid, as compared with 22.9 and 48.6 percent when the plants were inoculated with the parent lines S2 and C1, respectively. With few exceptions, on the varieties of sorghum susceptible to one parent line and resistant to the other, both intraspecific and interspecific hybrid lines proved to be intermediate in their virulence. Spur feterita (C.I. 623) proved to be immune from all of the parent and hybrid smut lines tested.

SUMMARY AND CONCLUSIONS

It is apparent from the results presented that physiologic forms of *Sphacelotheca sorghi* and *S. cruenta* may develop as a result of hybridization. Fusions readily occur between uninucleate sporidia of pathogenically distinct strains of *S. sorghi* and between sporidia of *S. sorghi* and *S. cruenta*, thus initiating the formation of diploid hyphae containing hereditary factors of two parental lines. Factors governing the sex of sporidia, the general morphology of the smut sori, the color of peridia, and the degree to which host plants may be stunted are inherited independently of each other. This means that considerable variation is possible in segregating progenies and accounts for the intermediate types produced as a result of interspecific hybridization. Since fusions can take place so readily in the laboratory, it may be assumed that such crossing may take place in nature. It is significant that a description of the general morphology of these intermediate types is in every way similar to the one given by Tisdale, Melchers, and Clemmer (*27*) for the appearance of their milo and feterita strains, and lends support to one of their explanations regarding the origin of those forms, namely, that they may represent hybrids between the two species *S. sorghi* and *S. cruenta*.

Obviously there is opportunity in the hybridization process for segregation and recombination of factors for pathogenicity, and as a result one might expect some of the progenies to differ in virulence from either of the parents. In the pathogenicity tests recorded here, hybrids were by no means equally virulent with parent lines. On Reed kafir intraspecific hybrids were more virulent than were parent lines, while interspecific hybrids were less virulent. In general, on the varieties of sorghum susceptible to one parent and resistant to the other, both intraspecific and interspecific hybrids were intermediate in virulence. Although the number of hybrid lines tested for pathogenicity was small, the results indicate clearly that pathogenically distinct strains may be produced as a result of hybridization.

It was suggested by Tisdale et al. (*27, p. 836*) that—

> There may be no sharp line of demarcation between *S. sorghi* and *S. cruenta*, as the descriptions in literature indicate. If a large number of collections of kernel smut were made from a large number of host varieties and under a wide range of climatic conditions throughout the world, it might be found that what has been described as two species simply represents the extremes in a series of variants.

At present nothing is known concerning the chromosomes and relatively little of the genetics of *Sphacelotheca sorghi* and *S. cruenta*. It is, therefore, difficult to make a definite statement regarding the relationship of these two fungi. The readiness with which species cross and the degree of sterility in the resulting hybrids constitute two criteria of relationship in the taxonomic system of higher plants. Of the two the degree of sterility of the hybrid has been considered the more useful index of relationship. Judging by these criteria, there is some evidence from the data recorded here that *S. sorghi* and *S. cruenta* are distinct species. Fusion between sporidia of what are considered *S. sorghi* and *S. cruenta* readily occurs, possibly indicating a close relationship in the taxonomic system. Of greater significance is the fact that on the majority of promycelia developed from F_1 hybrid chlamydospores of the S1–I–2, C1–I–2 cross, relatively few sporidia were formed, but instead peglike structures occurred which failed to develop either sporidia or hyphal threads. The writer is inclined to consider this failure to develop sporidia or hyphal threads from these segments as the consequence of genetic sterility. As sterility is the most characteristic feature of interspecific hybridization, these data are considered as evidence that *S. sorghi* and *S. cruenta* are distinct species.

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A NEMATOSIS OF AMSINCKIA CAUSED BY A NEW VARIETY OF ANGUILLULINA DIPSACI[1]

By G. STEINER, *senior nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture,* and C. E. SCOTT, *plant pathology specialist, College of Agriculture of the University of California*

INTRODUCTION

Although over 200 specific host plants of the bulb or stem nema, *Anguillulina dipsaci* (Kühn, 1858), are known, the discovery of a new one is always of interest. A new host may throw light on the origin of a pest in a given locality or on the possible source of reinfestation following the application of control measures, as well as on the problems of host transfers and the formation of races and varieties. Furthermore, the characteristic disease symptoms of the various host plants are of interest in indicating the pathological effects of the parasite.

The newly discovered host plant *Amsinckia intermedia* Fisch. and Mey. belongs to the borage family and is one of the commonest spring flowers in the interior valleys of California, where a great variety of plants is known to be infested with *Anguillulina dipsaci*. Among these are teasel, oats, garlic, alfalfa, clover, *Hypochaeris radicata*, *Crepis virens*, *Plantago lanceolata*, and various bulbs, such as narcissus, onion, hyacinth, etc. The host material described herein was collected in the spring of 1930 [2] from a small group of plants of *Amsinckia intermedia* growing along the roadside near Winters, Calif. In the spring of 1932 the disease was again observed on *Amsinckia* plants (1) in a vineyard at Planada, Merced County; (2) along a road adjacent to grainfields at Woodland, Madison Road, Yolo County; and (3) along a lane at the edge of a grainfield, Monticello, Napa County. It is not known whether these infestations were derived from such plants as teasel or were indigenous.

SYMPTOMS OF THE DISEASE

All portions of the plant above the ground may harbor the nema. The most outstanding symptom of the disease is found in the greatly enlarged fruits, transformed by the parasite into obvious galls. The diameter of a normal fruit is only about 3 mm, whereas the diameters of fruits transformed into galls may range from 5 to 10 mm. The diseased fruit is always located at the lower end of the inflorescence. The galls swarm with *Anguillulina dipsaci*—males, females, and larvae. Infested fruits are shown in figure 1.

ANGUILLULINA DIPSACI VAR. AMSINCKIAE N. VAR.

COMPARISON WITH OTHER FORMS

The *Anguillulina dipsaci* specimens described herein differ somewhat from both the form found in narcissus (*Narcissus* L.) and

[1] Received for publication Sept. 28, 1934; issued March, 1935.

[2] The collections were made by Grace M. Cole, assistant botantist, Division of Seed Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

potato (*Solanum tuberosum* L.), etc., and the form originally described by Kühn [3] from the teasel (*Dipsacus fullonum* L.), although they appear to be more nearly related to the latter. The question therefore arises as to the possible existence of varieties within *A. dipsaci*. The form from teasel is considered as the type, *A. dipsaci dipsaci*; the form living in narcissus, potato, etc., is then called *A. dipsaci* var. *communis* n. var.; and for the form under discussion the name *A. dipsaci* var. *amsinckiae* n. var. is proposed. A third variety recently [4] has been described as *A. dipsaci* var. *allocotus*. It has these characters: Vulva at 87 percent, very prominent because of an abrupt narrowing of body posterior to it; posterior uterine branch absent; distance vulva-anus about the same as tail length.

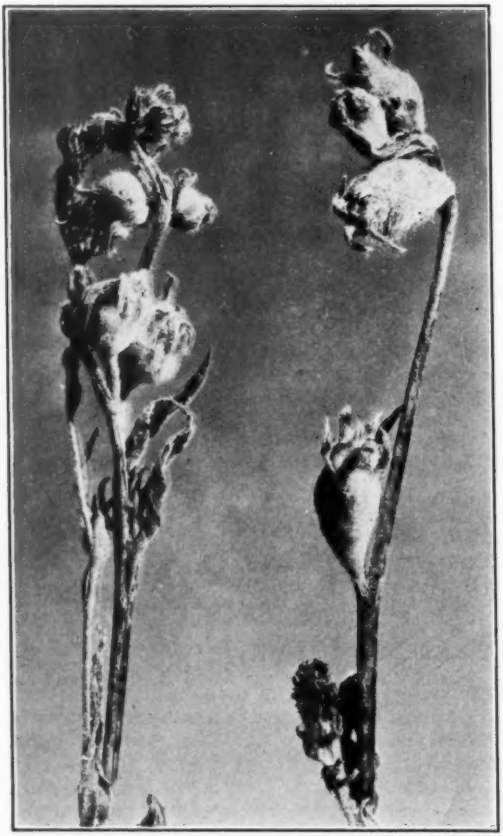

FIGURE 1.—Specimens of *Amsinckia intermedia* infested with *Anguillulina dipsaci* var. *amsinckiae*.

There is no intention here to make a final classification of the forms hitherto described under the name *A. dipsaci*, but only to call attention to certain differences in material heretofore considered morphologically rather uniform. The senior writer has seen material from teasel, but at the time did not pay special attention to the differences represented. These variations, however, will be apparent from a comparison of Kühn's original description with forms from narcissus, potato, etc., and with the specimens herein described from *Amsinckia*. Though showing some variation, the population found on *Amsinckia* was, on the whole, quite uniform.

The dimensions of *Anguillulina dipsaci* var. *amsinckiae* are very similar to those given by Kühn for the form from teasel, here called

[3] KÜHN, J. ÜBER DAS VORKOMMEN VON ANGUILLULEN IN ERKRANKTEN BLÜTENKÖPFEN VON DIPSACUS FULLONUM L. Ztschr. Wiss. Zool. 9: 129–137. 1858.

[4] STEINER, G. A NEW VARIETY OF THE BULB OR STEM NEMATODE, ANGUILLULINA DIPSACI, AND OTHER VARIATIONS IN THIS SPECIES. Helminthol. Soc. Wash. Proc. 1: 18–19. 1934.

Anguillulina dipsaci dipsaci. Transcribed into the decimal formula, Kühn's measurements are:

Female:	?	?	—	80.0	?	0.94–1.144 mm
	?	?	—	2.8	?	
Male:	?	?	—	M	93.3	1.036–1.269 mm
	?	?	—	2.4	?	

Unfortunately, his measurements for the esophagus are not correct, and are therefore controversial. In the form from *Amsinckia* the following dimensions were observed:

Female:	1.1	7.5	13.0	$^{70}89.7^{4}$	95.4	1.07–1.42 mm
	1.2	2.1	2.7	3.2	1.8	
Male:	1.3	8.9	14.0	^{68}M	93.5	1.04–1.30 mm
	1.3	2.1	2.6	3.7	2.4	

Anguillulina dipsaci var. *amsinckiae* is much thicker and the whole body is much more spindle-shaped than the type form, whereas the form from narcissus, potato, etc., is slenderer than the type form. Kühn gives 2.8 percent of the total length as the maximum diameter for the form from teasel. In *A. dipsaci amsinckiae* the maximum diameter is 2.2 to 3.9 percent of the total length—overlapping the maximum given by Kühn. Kühn gives the position of the vulva at 80 percent; in the variety *amsinckiae* the position of the vulva varies from 83 to 91 percent. Since a more detailed description of the type form from teasel is not available, for the present the diagnosis of the new variety must be based upon its greater body diameter and the more caudad position of the vulva.

DESCRIPTION OF NEW VARIETY

In *Anguillulina dipsaci* var. *amsinckiae* the cuticle shows extremely fine transverse striae, which are most pronounced near the head. This annulation, however, does not extend into the lateral fields, which seem to be smooth along their borders. The lateral fields consist of three distinguishable parts: a central one, measuring about one-fifth of the body diameter and bearing at least four fine longitudinal striae, and on each side of this an entirely smooth portion, somewhat wider than the central one. No traces of lateral membranes were seen. The intestinal cells are very large; there may be only 2 to 3 in a cross section. They are usually densely granulated; it is therefore almost impossible to distinguish individual cells in larval specimens. The rectum and the anal opening are fine, even obscure (fig. 2, *B*), indicating that little undigested matter is excreted. The oral spear is undoubtedly functional (fig. 3, *D*); the writers have seen it make short repeated thrusts, only the pointed part being protruded; apparently it serves chiefly for puncturing. The excretory pore is always well marked (fig. 3, *A*). The excretory duct is more heavily cuticularized at the end, and in certain specimens may be followed quite a distance back; the excretory cell, however, was not located.

The vulva of the female is extremely prominent (fig. 2, *B*); the opening forms a wide transverse slit. A reduced posterior branch of the uterus extends about two-thirds of the distance back to the anus. Thus the female apparatus is prodelphic, the ovary sometimes reach-

ing close to the beginning of the intestine; it is never reflexed. As shown in figure 2, *B*, eggs in the uterus of the female contained fully developed embryonic larvae that were moving around; but unsegmented eggs, as well as those in various stages of segmentation, were seen deposited. However, the fact that when specimens were taken from the plant unsegmented eggs were found in the watch glasses that served as containers should not be considered as proof of the oviparity of the form, since abortion may have resulted from the sudden immersion into water. In connection with the type species, Kühn states that he saw deposited both segmented and unsegmented eggs, as well as those containing embryos. He makes no statement as to the developmental stage within the female. In the variety from *Amsinckia*, the distance between vulva and anus is usually about 1⅛ to 1½ times the length of the tail. In the variety from narcissus this distance may be about 2 to 3 times the length of the tail (fig. 2, *C*).

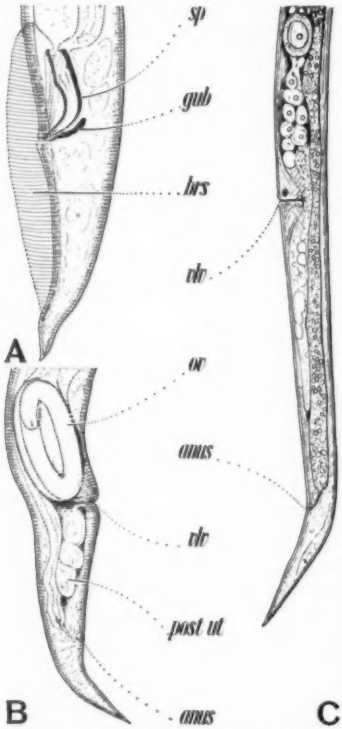

FIGURE 2.—*A*, Tail of male *Anguillulina dipsaci* var. *amsinckiae: brs*, Bursa; *gub*, gubernaculum; *sp*, spicula. × about 465. *B*, Posterior end of female *A. dipsaci* var. *amsinckiae: anus*, Anus; *ov*, egg; *post ut*, posterior branch of uterus; *vlv*, vulva. × about 250. *C*, Posterior end of female *A. dipsaci* var. *communis* from narcissus: *anus*, Anus; *vlv*, vulva. × about 250.

The male apparatus very closely resembles that described for the type. The testis may or may not have a reflexed end (fig. 3, *E* and *F*). In the present form the length of the spicula is about half the length of the tail (fig. 2, *A*). This is also true for the type. In the variety *communis*, however, the spicula are only about one-third or one-fourth the length of the tail (table 1). The bursa reaches close to the tail end, as in the type species from teasel, but differs from the form found in narcissus, potato, etc., here called variety *communis*, where it extends only part way down the tail. There is some variation in the manner in which it begins at its anterior end. In some specimens the edge of the bursa curves gradually down; in others it forms a right angle. In some of the specimens elongate crystals, most of which had rounded surfaces, were observed in the testis and its outlets (fig. 3, *B* and *C*). The nature and significance of these crystals are unknown. The late N. A. Cobb, who tested them optically, stated [5] that they are not birefringent. They were not seen in all specimens, and may have been pathological in nature or produced by death.

[5] Verbal communication.

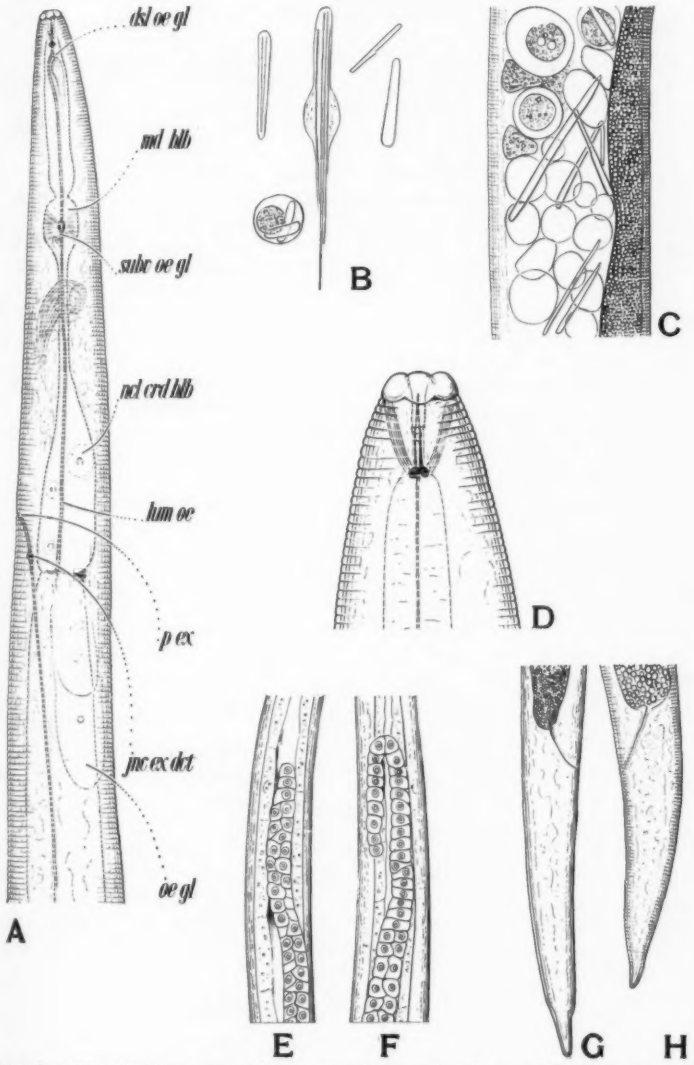

FIGURE 3.—*Anguillulina dipsaci* var. *amsinckiae*. *A*, Anterior portion of a female: *dsl oe gl*, Dorsal opening of an esophageal gland; *jnc ex dct*, junction of heavily and lightly cuticularized portions of excretory duct; *lum oe*, lumen of esophagus; *md blb*, medial bulb; *ncl crd blb*, large nucleus of cardiac (posterior) bulb; *oelg*, esophageal gland; *p ex*, excretory pore; *subv oe gl*, subventral opening of an esophageal gland. ×about 465. *B*, Various types of crystals from testis of male. Freehand sketch. *C*, Portion of male testis, showing crystals. ×about 530. *D*, Head of female, showing functional spear. ×about 1,430. *E*, Blind end of male testis, outstretched. ×about 250. *F*, Blind end of male testis, reflexed. ×about 250. *G* and *H*, Tails of two larval specimens, showing variation in shape. ×about 465.

TABLE 1.—*Comparative measurements of Anguillulina dipsaci showing differences in varieties*

Variety	Host	Authority for measurements	Average of maximum female body width (in percent of total length)	Position of vulva (in percent of total length)	Ratio of vulva to anus length to tail length	Ratio of spicula length to tail length
A. dipsaci dipsaci	Teasel	Kühn, 1858	2.8	80	(1)	1:2.27
A. dipsaci var. *amsinckiae*	*Amsinckia*	Senior writer	3.2	89.7	1:0.714	1:2.38
A. dipsaci var. *communis*	Narcissus	Ouboter, 1930	2.26	81	1:0.502	1:3.86
A. dipsaci var. *communis*	Onion	Ritzema Bos, 1888.	2.9	80	1:0.678	1:4
A. dipsaci var. *allocotus*	Unknown (found in soil).	Steiner, 1934	2.6	87	1:1.16	(2)

[1] Unknown. [2] No males seen.

DIAGNOSIS

Anguillulina dipsaci very close to the type from teasel as originally described by Kühn, in 1858, but differing in being thicker and more spindle-shaped, with an average maximum body diameter of 2.2 to 3.9 percent, and in having the vulva closer to the anus, that is, at 83 to 91 percent of the total length, the distance between the vulva and the anus being about 1⅕ to 1½ times the length of the tail.

ANABIOSIS

Since it is known that the type form of *Anguillulina dipsaci*, as well as the variety *communis*, is capable of withstanding adverse life conditions for a considerable length of time in a dormant stage, the variety *amsinckiae* was also tested in this regard. On the day it was received (May 7, 1930), part of the diseased material was placed, under absolutely dry conditions, in a refrigerator. From time to time the ability of *A. dipsaci* var. *amsinckiae* to revive was tested by putting small quantities of the infested tissue into water. Up to September 10, 1934, many of the pre-adult specimens revived at each test.

SUMMARY

Fruit galls of *Amsinckia intermedia* Fisch. and Mey., a native California member of the borage family, are described. The causal agent was found to be a new variety of *Anguillulina dipsaci*, the bulb or stem nema. The variety is described as *A. dipsaci* var. *amsinckiae*, and proof is given of its ability to revive after remaining dormant for at least 4 years and 4 months.

SOME MICROBIOLOGICAL ACTIVITIES AFFECTED IN MANURIAL CONTROL OF COTTON ROOT ROT[1]

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INTRODUCTION

A manurial experiment in progress since 1921 at the United States Field Station, Sacaton, Ariz., has demonstrated the efficacy of organic manures applied in deep furrows for controlling the cotton root-rot disease (*14*, *15*).[2] After the first application of organic material a definite reduction occurred in the area of cotton plants killed by the root-rot organism, *Phymatotrichum omnivorum* (Shear) Dug., and in succeeding years the disease was never severe in the treated plots although there was some fluctuation in extent of affected areas. The organic materials also had the effect of delaying the incidence of the disease, and most of the plants on the treated plots were able to mature a full crop before succumbing to it.

Experiments in root-rot infested areas outside of the irrigated region have failed to show such pronounced benefits from manures, but it is not reported that applications were made in deep furrows as they were in the Sacaton experiments. Several theories have been advanced in explanation of the marked results obtained in Arizona. The writers considered the following: (1) The organic acids from the decomposing manure reduce the alkalinity of the soil and make it less favorable for the fungus; (2) the evolution of ammonia in the manured plots inhibits or kills the fungus; (3) the stimulation received by the fertilized plants gives them greater resistance; (4) many roots are caused to develop superficially and are thus able to escape the fungus attack; (5) the manure increases the number and activities of competitive organisms which inhibit the growth and development of the root-rot fungus.

The last-named hypothesis was suggested to one of the writers in 1925 by the late K. F. Kellerman, then associate chief of the Bureau of Plant Industry. It has been supported by the work of Sanford (*23*) and of Millard and Taylor (*18*) on the control by green manuring of the potato scab disease caused by *Actinomyces scabies* (Thax.) Güs. Sanford (*23*) suggested that control of scab in some manured soils was due to the antibiotic qualities of certain soil organisms which influenced the development of *A. scabies*. Millard and Taylor (*18*) in 1927 showed that the effect of green manuring was to increase the number of saprophytic organisms which tended to choke out the parasitic scab-producing actinomyces (*A. scabies*). More recently Henry (*10*) found that the foot-rotting fungus of wheat, *Helminthosporium sativum* Pam., King, and Bak., was inhibited by the natural microflora of a black loam soil. During the last 4 years Henry (*11*), Broadfoot (*1*), Garrett (*8*), and Fellows (*7*) have published much

[1] Received for publication July 23, 1934; issued March 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 1105.

information on the antagonistic effect of natural soil microflora on the fungus *Ophiobolus graminis* Sacc., which causes the take-all disease of wheat. Fellows (7) found that applications of chicken manure, horse manure, alfalfa stems and leaves, boiled barley and oat mixtures, and potato flour, both in greenhouse and field, reduced the severity of take-all.

In the root-rot experiment at Sacaton, determinations of hydrogen-ion concentration of the soil were made on all of the plots involved in 1927, but there was no material difference in the pH values of manured or unmanured soils, all of which averaged from 7.8 to 8.2. Ammonia determinations made in 1932 and 1933 failed to show more than a trace of ammonia in the upper 2 feet of soil 10 weeks after application of the manure.

In recent studies in connection with the experiment on root rot control by organic manures, evidence was obtained which supports the theory that stimulation of soil organisms may have brought about conditions unfavorable for the root-rot fungus. A report of these investigations is presented in this paper.

CONTROL EXPERIMENTS WITH ORGANIC MANURES IN ARIZONA

The manurial experiment as conducted since 1923 comprises 2¼ acres divided into 9 quarter-acre plots. When the experiment was begun in 1921, only 8 plots were included, and manure was applied to only 1 plot, C2–19, which extended through the center of the infested area. The area of dead plants as mapped on this plot in 1920 was 71.7 percent, and the average percentage of area infested on this and the 3 other plots later manured was 66.8 percent. The area of dead plants on 4 alternate plots which received no organic manures throughout the period of the experiment was 57.3 percent of the whole. In 1923 about 12 tons per acre of manure was applied in furrows 10 to 12 inches deep on plots C2–17, 19, and 21, and 15 tons per acre of green alfalfa on plot C2–23. Plots C2–16, –18, –20, –22, and –24 were used as controls.

The same procedure was followed in subsequent years, except that the quantities of manure ranged from 12 to 14 tons per acre, and the alfalfa ranged from 8 to 15 tons per acre depending upon whether green alfalfa or spoiled alfalfa hay was used. In 1928 the manure was omitted from plot C2–19, and in 1929, C2–21 and C2–23 were not manured and were planted to alfalfa, while the corresponding control plots C2–22 and C2–24 were fallowed. In 1931 the Acala variety of upland cotton was substituted for the Pima Egyptian type, which had been grown previously, because of the greater resistance of the upland type to the root-knot disease, caused by nematodes, which had become prevalent throughout the entire block of nine plots.

Data on the percentage of area infested each season in these plots have been reported in previous publications (15, 16) for the years 1919 to 1930. The location and extent of the areas of dead plants killed by root rot at the end of the 1933 season is shown by diagram in figure 1 and by photograph in figure 2.

A count of plants dying from root rot was made at biweekly intervals in all of the plots during the year 1923 to 1933. The data obtained from the counts made in 1933 are given in table 1.

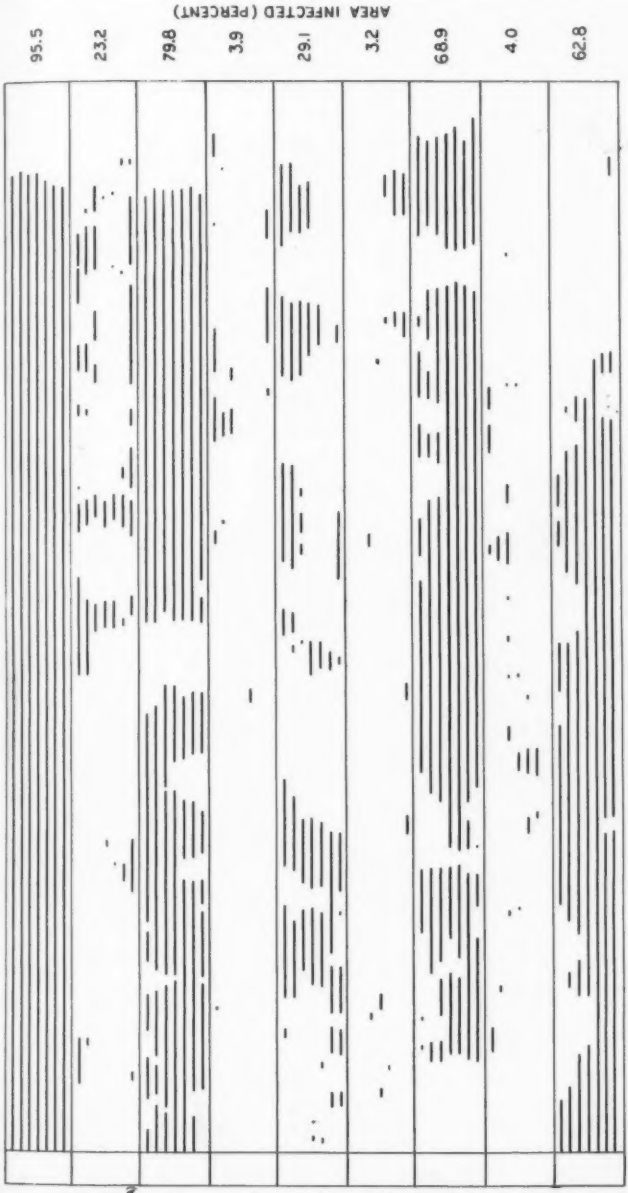

FIGURE 1.—Area infested with root rot in plots of Acala cotton in the manurial experiment at Sacaton, Ariz., 1933.

TABLE 1.—*Number of cotton plants killed by the root-rot disease during 2-week periods on 1/4-acre manured and control plots in 1933*

Date of count	Cotton plants killed under indicated treatment in plot no.—									Mean	
	C2-16 (unmanured)	C2-17 (manured)	C2-18 (unmanured)	C2-19 (manured)	C2-20 (unmanured)	C2-21 (manured)	C2-22 (unmanured)	C2-23 (manured) [1]	C2-24 (unmanured)	Unmanured plots	Manured plots
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
July 27	21	0	17	0	18	0	26	0	54	27	0
Aug. 10	97	4	129	1	53	1	210	5	299	158	3
Aug. 24	292	4	361	12	85	2	580	29	935	431	12
Sept. 8	292	7	369	10	107	12	506	88	719	398	29
Sept. 22	200	14	305	10	75	17	223	74	159	192	29
Oct. 9	296	14	254	2	83	11	90	43	92	163	17
Oct. 23	74	9	66	14	70	6	82	35	33	65	16
Total	1,272	52	1,501	49	491	49	1,617	274	2,291	1,434	106

[1] Manured with spoiled alfalfa.

CARBON DIOXIDE METHOD

Of the chemical methods for determining the activity of soil microorganisms in the decomposition of organic materials, evolution of

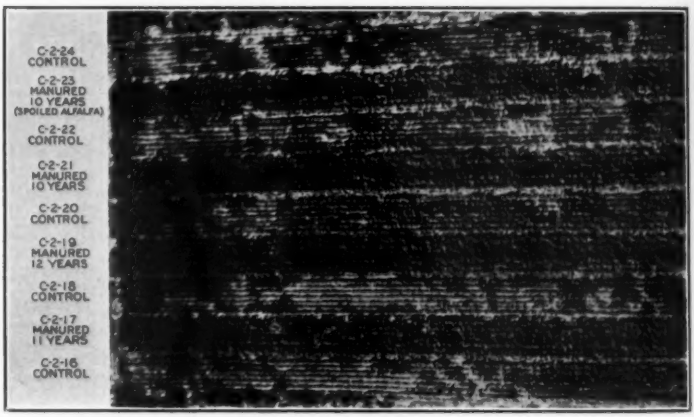

FIGURE 2.—Plots of Acala cotton in the manurial experiment on the control of root rot. Aerial photograph taken from an altitude of 800 feet, October 29, 1933. Note the four dark streaks representing the manured plots, on which the cotton plants are nearly all alive and green. The light areas represent the spots of dead cotton plants killed by root rot, and correspond to the areas shown by black lines in figure 1.

CO_2 has been found to be a good index (*12*, *22*, *24*, *25*). Several methods have been developed to measure the carbon dioxide evolved from soils, but no one method, of those considered by the writers, appeared entirely satisfactory for conditions in the manured and unmanured soils under study. It seemed desirable to test the soils under natural conditions. The lack of suitable facilities for aspiration made it impracticable to operate in the field, however, so the feasibility of conveying large cores of undisturbed soil to the laboratory was tested. By using this procedure, a method was developed which consisted essentially of combined features from the methods described by Neller (*20*), Lundegårdh (*17*), Heck (*9*), and Humfeld (*13*).

The soil samples were obtained in sections of iron pipe as undisturbed cylindrical cores 30 cm long and 19 cm in diameter, with an average moist weight of 14.9 kg. After the bottoms were sealed over with paraffined paper, the cylinders were placed in closed chambers so that air could be passed over the surface of the soil sample, 283.5 cm^2 in area, rather than through it, which procedure, according to Potter and Snyder (*21*), more closely duplicates field conditions.

The apparatus comprised a cleaning train, a sealed glass chamber for the soil samples, an absorption unit, and a source of suction (fig. 3). A basement room was used as a laboratory in order to reduce the daily range between maximum and minimum temperatures. During the course of the experiment the range was seldom greater than 3°.

The cleaning train was similar to the one used by Heck (*9*), except that a barium hydroxide tower was not included.

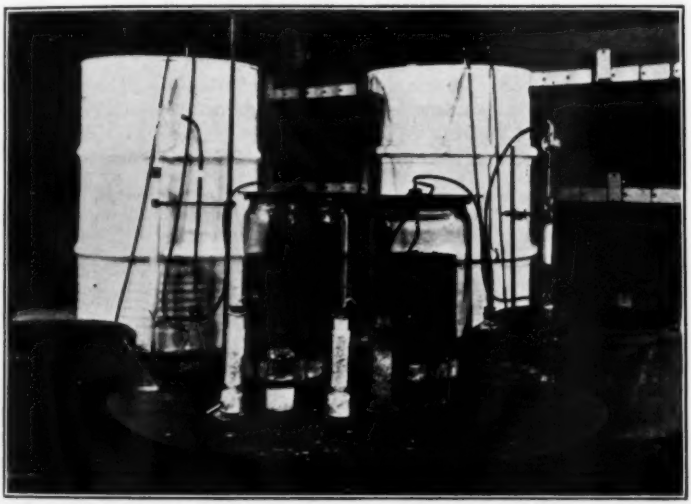

FIGURE 3.—Apparatus for determining the rate of evolution of carbon dioxide from large samples of manured and unmanured soils in the root-rot control experiment. The soil samples are within the large glass jars in the foreground.

The soil-sample chamber was a glass jar 45 cm deep and 23 cm in diameter. A glass cover fitted with a 2-hole rubber stopper was sealed over the top with wax after the soil sample was in place. In operation the air from the cleaning train entered the jar at the top and was withdrawn through a tube with an opening near the bottom of the jar.

After leaving the jar, the air was drawn through the absorption unit, a modification of Heck's absorption tower. It consisted of a glass tube, 25 cm long and 25 mm in diameter, containing glass beads and provided at the bottom with two tubes, one for the withdrawal of the solution and the other for the air intake. Fifty cubic centimeters of N/4 potassium hydroxide was used to absorb the carbon dioxide from the soil chamber. A Kjeldahl bulb at the top of the tower prevented any carry-over of the solution. At 24-hour intervals the potassium hydroxide was washed from the Kjeldahl bulb and the

bead tower and titrated with N/4 hydrochloric acid, with phenolphthalein as the indicator.

Suction to pull the air through the system was supplied by a sealed siphon barrel made from a gasoline drum. The air current was regulated to flow about 50 to 75 l a day. The amount of air used each day was measured, but as the fluctuations which occurred had no apparent influence on the carbon dioxide, the measurements are not presented.

Two units of the apparatus were operated simultaneously, one with a soil sample from a manured plot and the other with a sample from an adjacent control plot. Each pair of samples was aspirated for a period ranging from 5 to 7 days, with titrations of the absorbing solution each 24 hours. The results from only 5 days for each sample were used in computations. Soil-moisture determinations were made when the samples were taken, and all but the first pair of samples were weighed.

Results

The plot treatment, the weight, the moisture content, and the date of collection of soil samples, and the average number of milligrams of CO_2 evolved per kilogram of soil are shown in table 2. The weights of the samples from plots C2–18 and C2–19 were estimated.

Table 2.—*Soil treatment, date of sampling, moisture percentage, and average number of milligrams of CO_2 evolved daily per kilogram of soil from manured and unmanured plots*

Plot and treatment	Date of sampling	Wet weight	Dry weight	Moisture (dry basis)	Average of CO_2 evolved daily per kilogram of dry soil
		Kg	*Kg*	*Percent*	*Mg*
C2–16, unmanured	July 8	14.9	13.09	13.9	6.60
C2–17, manured 11 years	do	14.5	12.85	12.8	14.37
C2–18, unmanured	June 29	15.0	12.9	16.3	8.78
C2–19, manured 12 years	do	15.0	12.9	16.3	14.57
C2–20, unmanured	July 15	15.9	12.78	24.4	[1] 11.75
C2–21, manured 10 years	do	15.9	13.00	22.3	[1] 15.65
C2–22, unmanured	July 22	14.2	12.39	14.6	9.14
C2–23, manured 10 years with spoiled alfalfa	do	13.8	12.32	12.0	11.67

[1] Average of two 5-day runs on different samples from same plot.

The range of moisture was from 12.0 to 24.4 percent, but neither of these figures represents extreme dryness or wetness for the type of soil involved, which has a moisture equivalent of about 20 percent. From the data obtained, it would not appear that small differences in the percentages of moisture are important in influencing the evolution of carbon dioxide.

Two samples each were run for plots C2–20 and C2–21, with an interval of 18 days between samplings. The results from both pairs are presented as averages in table 2.

The results as calculated on the basis of grams of CO_2 per square meter of soil are given in table 3, in which the results from the two samples from plots C2–20 and C2–21 are presented separately. It will be noted that only one sample, C2–20, from an unmanured plot exceeded the lowest manured plot, C2–23, in amount of carbon dioxide evolved. The 5-day average for C2–20 in the first test was 5.81 g of

CO_2 per square meter of soil surface, while for C2–23 it was 5.07 g. The second sample for C2–20 averaged 4.61 g of CO_2. The results from the two samples from the manured plot C2–21 are in close agreement, averaging, respectively, 6.9 and 7.15 g of CO_2.

It seems significant that the one plot manured with spoiled alfalfa hay showed a lower rate of carbon dioxide evolution and a higher rate of root-rot kill (fig. 1) than the three plots to which corral manure was applied.

TABLE 3.—*Grams of CO_2 per square meter of soil surface evolved daily for 5 days from samples of soil from manured and unmanured plots* [1]

Day	CO_2 per square meter of soil surface from indicated treatment of plot no.—									
	C2–16 (unmanured)	C2–17 (manured 11 years)	C2–18 (unmanured)	C2–19 (manured 12 years)	C2–20 (unmanured)	C2–21 (manured 10 years)	C2–20 (unmanured)	C2–21 (manured 10 years)	C2–22 (unmanured)	C2–23 (manured 10 years with spoiled alfalfa)
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
1	3.13	6.66	2.96	5.75	6.98	7.26	5.64	7.72	4.41	5.50
2	2.89	6.28	2.57	6.91	5.89	6.91	5.43	7.27	3.81	5.04
3	3.39	6.38	2.43	6.52	5.33	6.70	4.13	7.16	4.83	5.40
4	2.82	6.60	2.15	6.52	5.71	7.19	4.23	6.38	4.16	5.01
5	3.00	6.63	3.03	7.44	5.15	6.45	3.63	7.23	2.75	4.41
Total	15.23	32.55	13.14	33.14	29.06	34.51	23.06	35.76	19.96	25.36
Average	3.05	6.51	2.63	6.63	5.81	6.90	4.61	7.15	3.99	5.07

[1] These data were computed from those used in calculating the CO_2 evolved per unit of weight (table 2).

CHOLODNY'S SLIDE METHOD

In order to compare the populations of micro-organisms in the manured and unmanured plots, it seemed desirable to use some method by which the relative abundance of organisms in the various taxonomic groups could be estimated. It has been reported by Waksman (*25*) and others that the addition of organic matter increases the bacteria and actinomycetes in the soil but that the increase in the bacterial population after manuring is of short duration. It is known also that fairly rapid changes in the type and number of organisms occur with the successive stages of decay and with climatic changes. The progress of the season, the influence of temperature, irrigation, and the chemical and physical changes in the soil effected by the decomposition cycles undoubtedly are reflected in the composition of the soil flora. Regardless of these changes, however, even rough or approximate methods might distinguish differences in the soil population of soils which differ greatly in organic content, as did those under study.

During the summer of 1932 a preliminary attempt was made to investigate this problem by the culture-plate method. The technical difficulties and the shortcomings of this method, pointed out by Conn (*4, 5*) caused it to be abandoned. The direct microscopic method devised by Cholodny (*2*), with slight modification (*6*), was found to be more useful and was adopted.

Preparations obtained by the above-mentioned method show bacterial colonies, hyphae of fungi and actinomycetes, and occasionally protozoa and nematodes (fig. 4). It is suggested that the microslide becomes coated with the soil solution in the same way as quartz

grains do. Nearby micro-organisms and those which spread through the soil come in contact with the slide and grow along its surface, adhering to it because of the surface tension of the film of moisture.

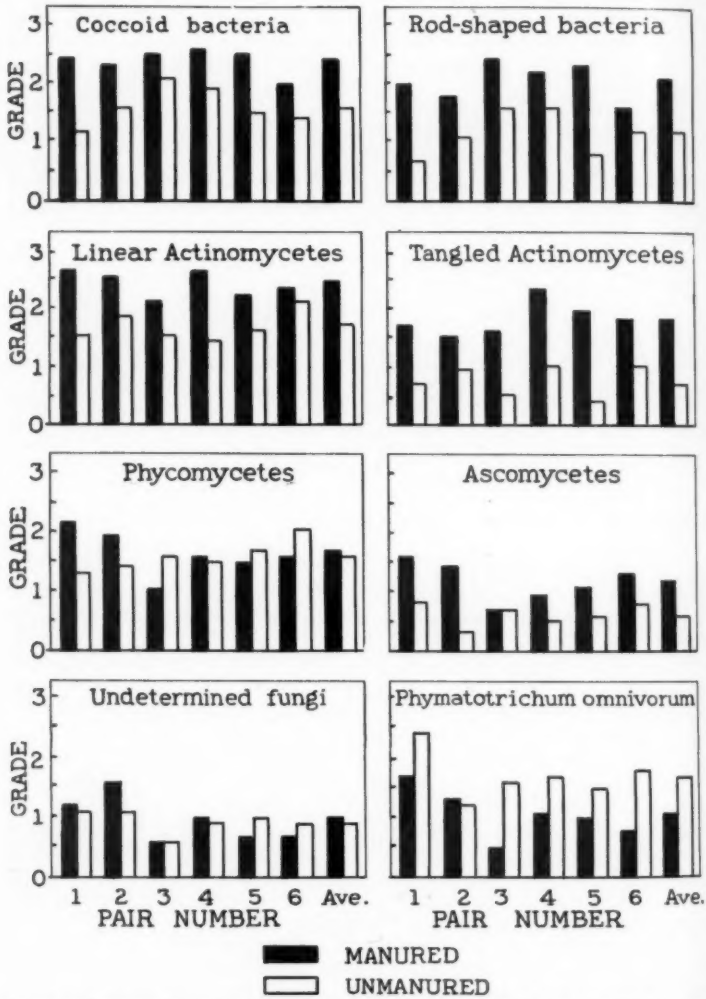

FIGURE 4.—Mean grades indicating density of certain microfloral populations as estimated from microslides exposed in manured and unmanured plots in a root-rot control experiment. Grade 0, absent; 1, few; 2, abundant; 3, profuse.

The slide technic is selective to some extent. For example, in a series of exposure periods of different lengths at the beginning of the season, it was observed that the complex of organisms on the slides varied with the length of the exposure period. Slow-growing forms

appeared only after long exposure, by which time the rapidly growing forms had partially disintegrated. It appeared, however, that an exposure period of 9 to 10 days was a good modal point, at least a standard for one type of comparison of the general population, and this was adopted for these studies.

In all, 120 microscope-slide preparations were made. Each manured plot and an adjacent unmanured plot made up a pair for comparative purposes. Ten slides were exposed simultaneously in each plot of the different pairs. Twenty slides were arranged in 2 rows, about 10 feet apart and extending across the 2 paired plots. The slides were spaced at 3½-foot intervals in the rows, giving a total of 10 in each plot. The various sets of exposures were made between July 14 and October 9, 1933. Soil temperatures and humidity were not greatly different for the treated and untreated plots, although other soil conditions no doubt varied, being influenced by the decomposition of the manure on the one hand and by the progress of the root-rot disease on the other. Exposure of the slides usually began on the third day after an irrigation.

For examination the slides were selected at random without reference to their identification number or to their location. This avoided the possibility of prejudice and distributed the errors of estimating. The entire surface of each slide was examined at magnifications 88 and 400. Population counts were made on the basis of taxonomic groups and were recorded according to an arbitrary standard of grades. Grading was on the basis of the approximate area of the slide occupied by each group. These grades and their symbols were as follows: Absent, 0; present but not abundant, 1; abundant to very abundant, 2; very abundant to profuse, 3. The following intermediate grades were also designated: 1.3, 1.6, 2.3, 2.6, and 3.3. A record was made for each slide and the mean average of the grades was computed for each group of 10 slides which had been exposed simultaneously in the same plot.

Observations

For comparison, the average data for adjacent manured and unmanured plots are arranged as pairs in table 4. The data are comparable only for exposures made on the same dates. The data for grade comparisons are the means of 6 to 10 slides for each of the manured and unmanured plots.

TABLE 4.—*Means and statistical constants of the grades indicating the density of microfloral populations in manured and unmanured plots in a root-rot control experiment, 1933*

Pair no.	Exposure			Treatment	Slides examined	Grade,[1] indicating density of population of—							
	Period	Days	Plot			Coccoid bacteria	Rod-shaped bacteria	Linear actino-mycetes	Tangled actino-mycetes	Phyco-mycetes	Ascomy-cetes	Undetermined fungi	*Phymatotrichum omnivorum*
		Number	*No.*		*Number*								
1	July 14–24	10	C2-17	Manured	10	2.4	2.0	2.6	1.7	2.2	1.6	1.2	1.7
			C2-16	Unmanured	[2] 9	1.2	.7	1.5	.7	1.3	.8	1.1	2.4
2	July 31–Aug. 9	9	C2-19	Manured	10	2.3	1.8	2.5	1.5	1.9	1.4	1.6	1.3
			C2-18	Unmanured	10	1.6	1.1	1.8	.9	1.4	.3	1.1	1.2
3	Aug. 23–Sept. 1	9	C2-21	Manured	[2] 7	2.5	2.4	2.1	1.6	1.0	.7	.6	.5
			C2-22	Unmanured	[2] 9	2.1	1.6	1.5	.5	1.6	.7	.6	1.6
4	do	9	C2-23	Manured	10	2.6	2.2	2.6	2.3	1.6	.9	1.0	1.1
			C2-24	Unmanured	10	1.9	1.6	1.4	1.0	1.5	.5	.9	1.7
5	Sept. 22–Oct. 2	10	C2-21	Manured	10	2.5	2.3	2.2	1.9	1.5	1.1	.7	1.0
			C2-22	Unmanured	10	1.5	.8	1.6	.4	1.7	.6	1.0	1.5
6	Sept. 30–Oct. 9	10	C2-19	Manured	[2] 6	2.0	1.6	2.3	1.8	1.6	1.3	.7	.8
			C2-20	Unmanured	[2] 6	1.4	1.2	2.1	1.0	2.1	.8	.9	1.8
Mean±P. E.:													
All manured plots[3]					53	2.4±.05	2.1±.05	2.4±.01	1.8±.08	.7±.06	1.2±.07	1.0±.06	1.1±.08
All unmanured plots[4]					54	1.6±.05	1.2±.05	1.7±.05	.7±.06	1.6±.05	.6±.6	.9±.07	1.7±.10
Difference of means± P. E.						.8±.07	.9±.07	.7±.05	1.1±.10	.1±.08	.6±.09	.1±.09	−.6±.13
Difference of means÷ P. E.						11.4	12.9	14.0	11.0	1.2	6.7	1.1	4.6

[1] Significance of grades: 0, absent; 1, few; 2, abundant; 3, profuse.
[2] The remainder of the group of 10 slides was lost or destroyed in manipulation.
[3] All data on manured plots used as one array.
[4] All data on unmanured plots used as one array.

The data in table 4 and the diagrams in figure 4 show that both the coccoid and the rod-shaped bacteria occurred in much greater abundance in the manured than in the unmanured soil. This also applies to the two types of actinomycetes, although the occurrence of these latter was not quite so consistent as that of the bacteria. Fungi other than *Phymatotrichum omnivorum* were only slightly more abundant in the manured than in the unmanured soils. One exception was that there were more phycomycetes in the manured plot, C2–17, of pair 1 than in the unmanured plot, C2–16. For the most part probable errors indicate that differences in fungus populations were not significant. There was a greater similarity in the microflora of manured plots C2–21 and 23 and their companion unmanured plots, C2–22 and 24, than occurred in the other pairs. This may have been due in part to the large numbers of dead cotton roots which were present in the unmanured soil when these exposures were made and which may have stimulated the production of saprophytic organisms.

Table 4 shows the mean grades and their statistical constants computed for all slides exposed in manured and unmanured soil. The means were computed from the 107 slides as originally classified, the figures for the manured soil being treated as one array, and those for the unmanured soil as another. Six of the eight groups of organisms that were being estimated occurred in significantly greater abundance in manured than in unmanured soil. *Phymatotrichum omnivorum* was the only fungus that was more abundant in the unmanured soil. The differences in the relative abundance of phycomycetes and miscellaneous undetermined fungi in the treated and untreated plots were not great enough to appear significant.

Ten slides were exposed on plots C2–21 and C2–22 at the end of August and again at the end of September (pairs 3 and 5, table 4). A comparison of the grades for population density in each of these plots on these dates indicates that there was a greater variation of population in the unmanured than in the manured soil. This was true particularly of bacteria and actinomycetes.

A number of cases of what appeared to be parasitism of *Phymatotrichum omnivorum* hyphae by other organisms (fig. 5, *A* and *B*), were observed. Of 54 slides from unmanured soil, this apparent parasitism was noted on 5; while of 53 slides from manured soil it was noted on 19.

DISCUSSION

The data in table 1, supported by similar data from previous years and the evidence in figures 1 and 2, show conclusively that the manurial treatment had some influence in reducing the damage to the cotton crop on the plots to which it was applied. The collateral cultural treatments, including the deep furrowing in winter, were the same for all plots. With the two chemically different organic materials (alfalfa hay and corral manure) giving similar results on control of the root-rot disease, it seemed reasonable to consider the populations of soil organisms and the processes which they bring about as responsible factors. The data in tables 2 and 3, showing the differences in evolution of CO_2 from manured and unmanured soil, may be taken as conclusive evidence that the soil organisms were more abundant and were bringing about chemical changes more rapidly in the treated soil.

The work of Neal and Wester (*19*), as well as that of the writers, shows that the growth of the root-rot fungus is restricted or inhibited in an atmosphere consisting of 50 percent or more of CO_2. The writers made no attempt to determine the CO_2 content of the soil atmosphere in the manured plots, but it seems improbable that it reached the proportion of 50 percent. Waksman (*25*) reports an instance where the soil atmosphere, after green manuring, consisted of approximately 12 percent CO_2, and Clements (*3*) records a case where the soil atmosphere was 14 to 21 percent CO_2 in plots that had been manured. The root-rot fungus is not restricted by these proportions of CO_2 in the laboratory.

An examination of the data in table 4 reveals the fact that the root-rot fungus *Phymatotrichum omnivorum*, was the only organism recorded which appeared in greater abundance on the slides in the

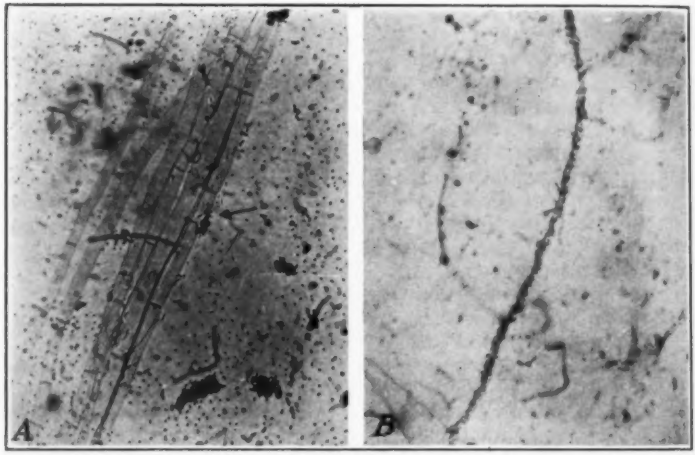

FIGURE 5.—Hyphae of *Phymatotrichum omnivorum* and bacteria which developed on slides exposed for 9 to 10 days in the soil of manured plots of a root-rot control experiment. The concentration of the bacteria on the hyphae suggests that they may be parasitic. *A*, Hyphae of *Phymatotrichum omnivorum* forming a strand, associated with numerous rod-shaped bacteria. The arrow points to a colony of bacteria on a hyphal cell. *B*, Bacteria covering a shrunken hypha of *Phymatotrichum omnivorum*. ×500.

unmanured plots. However, this fungus was by no means rare in the manured areas. When the manure treatment was suspended and alfalfa was planted for 1 year on plots C2–21 and –23 in 1929, it was demonstrated that the root-rot fungus, though reduced in amount, still existed in parts of the soil and was capable of rapid destruction of the new host crop. King and Loomis (*16*) referred to the inhibitory action of specific fungi and bacteria on the growth of *P. omnivorum* in cultures. This behavior is noticed particularly in connection with fermenting types of bacteria which take part in the preliminary decomposition processes in fresh plant tissues.

When all data and observations are considered collectively, the conclusion seems justified that the growth and parasitic activity of *P. omnivorum* in the manured plots were restricted by the presence of a dense population of other organisms actively engaged in the decomposition of bulky organic material with resultant byproducts

probably unfavorable for the growth of a fungus of the root-rot type. Whether one specific organism or a group of organisms was responsible for the inhibitory effects can be determined only by extensive investigations. The writers recognize the need of further study before the theory of control by competitive soil organisms can be regarded as proved.

SUMMARY AND CONCLUSIONS

The effectiveness of organic manures in controlling cotton root rot under irrigation conditions has been demonstrated by an experiment which has been conducted at the United States Field Station, Sacaton, Ariz., since 1921.

In 1933 an investigation was made to compare some of the microbiological activities in 4 quarter-acre plots which had received applications of manure for several years with that in 5 alternate plots of the same size which received no manure.

Determinations were made of the CO_2 evolved from large samples of soil obtained as cylindrical cores from each plot. In the choice and arrangement of the apparatus used in these determinations an effort was made to approximate field conditions in the laboratory.

In all determinations the samples from the manured plots evolved CO_2 at a greater rate than those from the unmanured plots. In comparisons between adjacent plots the rate of CO_2 evolution from the manured plots ranged from 19 to 152 percent greater than from unmanured plots, and the results indicate a greater microfloral activity as a result of the increased content of decomposing organic matter.

A modification of Cholodny's method of direct microscopic examination was used to obtain a measure of the relative abundance of different organisms in adjacent manured and unmanured plots. Clean microscope slides were exposed in the soil for several days; the various organisms which became attached to the slides were "fixed", and their relative density was measured by a system of grades based on the area occupied by each.

Bacteria and actinomycetes in various forms and most fungi were more abundant on the slides exposed in the manured plots. The root-rot fungus, *Phymatotrichum omnivorum*, was more abundant on the slides exposed in the untreated plots.

The tests show that the population of many organisms is greatly increased by large applications of organic matter to the soil. The rapid and prolonged reduction of root-rot activity on the manured plots suggests that the dense population of organisms engaged in breaking down the organic materials developed a soil condition temporarily unfavorable for the growth and activity of the root-rot fungus.

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A STUDY OF THE COTTON ROOT-ROT FUNGUS (PHYMATOTRICHUM OMNIVORUM) IN THE SOIL BY THE CHOLODNY METHOD [1]

By E. D. EATON, *junior pathologist*, and C. J. KING, *agronomist, Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Progress in soil microbiology has been handicapped by the lack of methods which give a true picture of the numbers, activities, and relationships of the organisms under field conditions. The culture-plate method is not accurate for judging the activity of soil fungi, since it affords information principally as to the number and kinds of spores present. The earlier microscopic methods of direct examination also fail to show the filamentous organisms in their true abundance. Those engaged in studying the fungus *Phymatotrichum omnivorum* (Shear) Duggar, the cause of the cotton root-rot disease, realize the need for obtaining a better knowledge of how this fungus maintains its existence as a soil inhabitant, and of the relationships which exist between it and associated organisms. The writers have found the procedure described by Cholodny [2] for the direct microscopic study of soil microflora well adapted for obtaining information on the biology of this important fungus. By using a slight modification of Cholodny's technic it was found practicable to make useful observations on the early-season growth of the fungus, on its occurrence independent of plant roots in cultivated and clean fallow soils, and on the type and abundance of associated microflora.

METHOD

The Cholodny technic consists in exposing standard microslides in the soil under field conditions. The procedure is simple, and requires only a little care in placing and removing the slides. Cholodny placed the slides in a shallow trench with one side pressed against the vertical face of the undisturbed soil, and with one end level with the soil surface. The soil region of greatest interest in root-rot investigations is from 6 to 18 inches below the surface. This made it necessary to devise a holder for placing and removing the slides. The holder is a modified stake made from a board 2½ to 3 feet long and 2 to 3 inches wide, with thin wooden strips tacked near the edges on one side to act as a retaining groove for the slide (fig. 1). By varying the depth at which the stake is placed and the location of the slide in the groove, placement may be at any depth from about 1 to 30 inches. It is often desirable to use more than one slide in a holder; in such cases both stakes and slides are numbered for convenient location and identification. The holders should be well coated with a water-resistant paint, preferably a nonturpentine mixture.

[1] Received for publication July 23, 1934; issued March 1935.

[2] CHOLODNY, N. UEBER EINE NEUE METHODE ZUR UNTERSUCHUNG DER BODENMIKROFLORA. Arch. Mikrobiol. 1: 620–652. 1930.

The writers found cellulose acetate dissolved in acetone very satisfactory. This paint prevents the contamination of the soil by wood-destroying fungi, yet does not repel or inhibit soil micro-organisms by turpentine fumes or leachings. Before exposure the slides should be numbered with a carborundum pencil, cleaned in acid, water, and alcohol and thoroughly dried. The slides used by the writers were 25 by 75 mm in size, made of hard crown glass which showed a slight greenish tint.

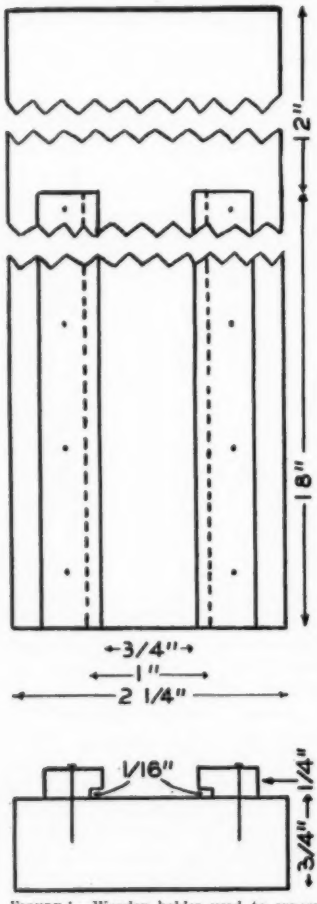

FIGURE 1.—Wooden holder used to expose microslides in the soil.

In preparing to expose a slide a hole is dug about 1 foot wide and slightly deeper than the desired depth of placement. A slide is inserted in the groove of a holder and set for the depth desired. One face of the hole is cut smooth with a large knife, and the side of the stake on which the slide is attached is pressed firmly against this surface so that contact is made between the soil and the slide. It is important that both placement and removal be performed with care to prevent making a smear on the slide. After placing the slide in position the hole is refilled, the soil being packed to about the density of that surrounding. After exposure for about a week, or longer if desired, the slide is removed by reversing previous operations. As soon as possible after removal the slide is dried over a flame or hot plate, stained for 1 minute in boiling rose bengale solution, and washed rapidly in water. After drying, the slide is ready for examination.

Preparations obtained by this method show bacterial colonies, hyphae of fungi and actinomycetes, and occasionally protozoa and nematodes. It has been suggested that the microslides, somewhat like quartz grains, become coated with the soil solution. Nearby micro-organisms and those which spread through the soil come in contact with the slide and grow along its surface, adhering to it by the film of moisture. Before using the stakes for other exposures they should be thoroughly cleaned to avoid contamination by the transfer of organisms.

RESULTS

Preliminary tests have shown that it is possible by the method just described to ascertain for a specific organism the time of year at which

growth begins and the depth at which activity occurs, and also its horizontal distribution. Associated organisms and to some extent their interrelations are also shown. The frequency with which

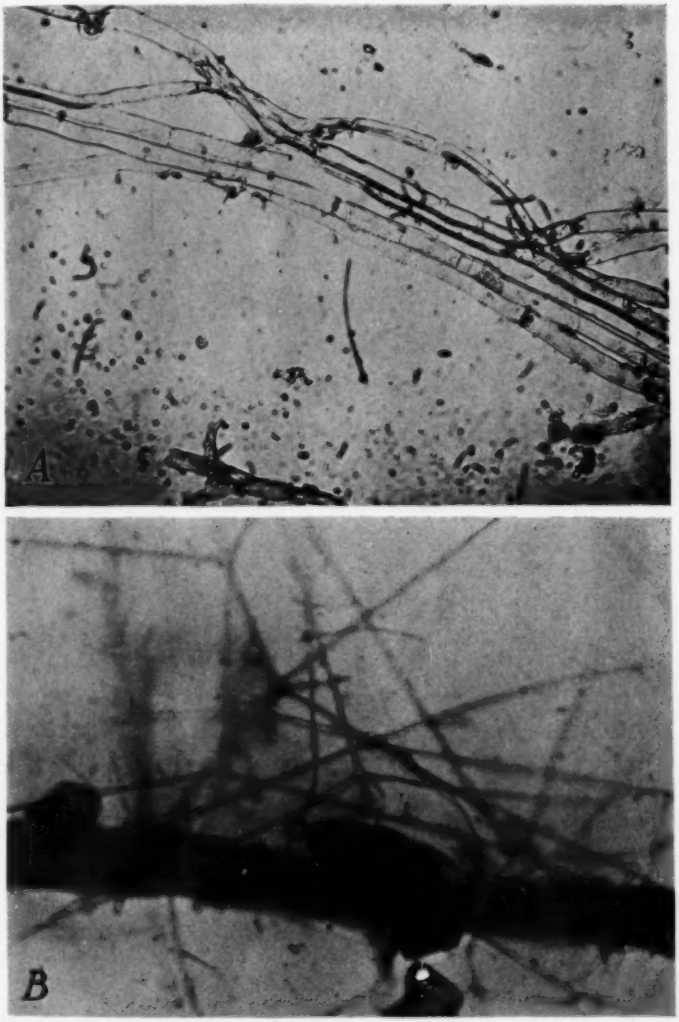

FIGURE 2.—Mycelium of *Phymatotrichum omnivorum* as observed by direct exposure of microslides in the soil. *A*, Hyphae beginning to develop into strand form. × 500. *B*, A small strand and acicular hyphae. × 500.

individual organisms and colonies occur on the slides may be assumed to be a rough index of the frequency of occurrence in the soil.

The first application of this method revealed hyphae of *Phymatotrichum omnivorum* growing in the soil of a cotton field known to be

infested. Observations showed single hyphae of the large barrel-shaped cell type, small strands formed by the further growth and development of the former (fig. 2, *A* and *B*), and fine hyphae composed

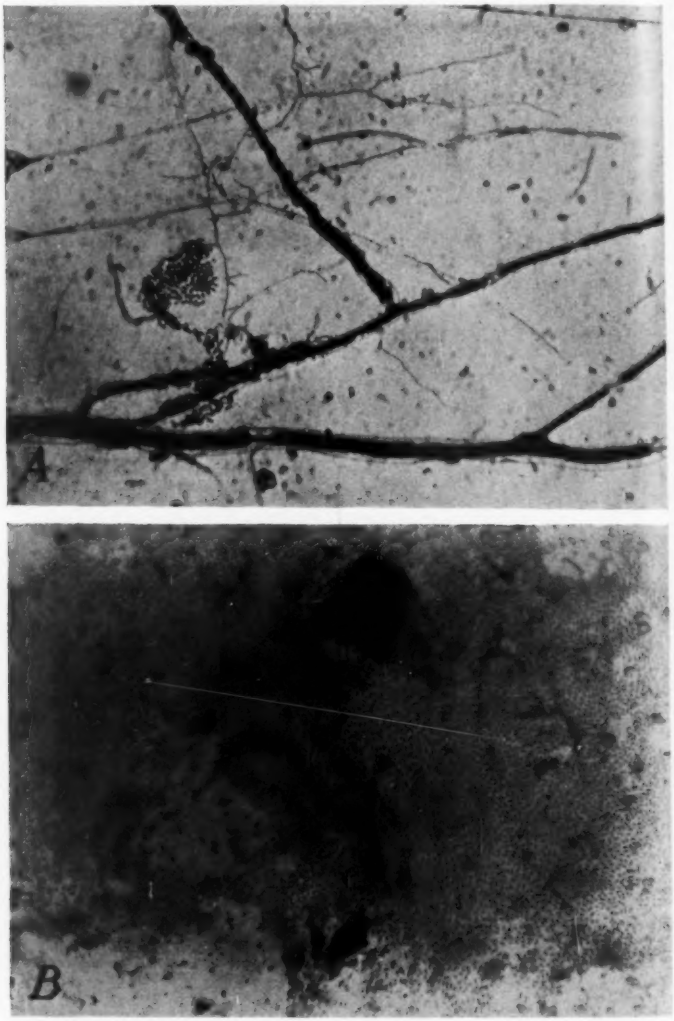

FIGURE 3.—Organisms commonly found in association with *Phymatotrichum omnivorum* by direct exposure of microslides in a root-rot-infested cotton field. *A*, Mold hyphae, actinomycetes, and bacteria. ×500. *B*, A colony of rod-shaped bacteria. × 500.

of long, narrow cells. Occasionally acicular hyphae were observed in connection with the strand forms. Growth of *P. omnivorum* first occurred on the slides in the week of May 22–29, 1933, although at

that time the cotton plants adjacent to the slide locations did not show indications of root-rot infection, nor was infection apparent during the succeeding 45 days. There was nothing to suggest that the hyphal growth was attached to the roots of host plants. A number of slides exposed in an infested area maintained for some months as a clean fallow also showed unmistakable growths of *P. omnivorum*. In either of these cases there is a possibility that the growth may have come from germinating sclerotia.

Organisms other than *Phymatotrichum omnivorum* obtained on the slides included *Rhizoctonia* sp., *Trichoderma* sp., *Aspergillus* sp., and other fungi, numerous actinomycetes, and bacteria of many types (fig. 3, *A* and *B*).

DISCUSSION

The technic described promises to facilitate the study of the biology of *Phymatotrichum omnivorum* by making possible the direct observation of mycelial activity in the soil under natural conditions. Such information may be of use in control studies by indicating the initiation of activity in the spring and the efficacy of cultural or chemical treatment. Some indication of its value was shown by the observation of active *Phymatotrichum* hyphae in areas of clean fallow. The method brings into view the outstanding features of the general soil flora. It has limitations, however, since identification of the micro-organisms as they occur on the slides is impossible except in rare cases, and it is not possible to make a population census on the basis of units of soil volume or weight. However, crude comparisons of soil populations at different times and at different locations can readily be made, and decided changes in population may be detected.

SUMMARY

A method is described by which the growth and development of the mycelium of the root-rot fungus *(Phymatotrichum omnivorum)* in the soil under natural field conditions may be observed.

Preliminary observations show that: (1) *Phymatotrichum* mycelium was active in the soil at least 6 weeks before the first symptoms were observed on nearby cotton plants; (2) the growth of the mycelium in the soil apparently was not connected with roots; and (3) the mycelium developed in a clean fallow.

Suggested uses of the method in investigations of the root-rot fungus are (1) for biological and control studies, (2) as a means of ascertaining the presence of the fungus in fallow lands, and (3) in studies of its interrelations with other organisms.

NITROGEN FIXATION STUDIES WITH FUNGI AND ACTINOMYCES[1]

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INTRODUCTION

The claim that many species of fungi can use atmospheric nitrogen as the sole source of this element has been made many times during the past 50 years and about as frequently denied. The status of our knowledge on the subject is still such that positive statements regarding the nitrogen-fixing ability of few, if any, of these organisms can be made. Only a relatively few of the many species and strains of the true fungi and closely related forms have been tested adequately. The genus *Aspergillus*, and to a lesser extent *Penicillium*, among the true fungi, have received the most study. Considerable attention has also been given to certain of the fungi which are capable of producing mycorrhiza on the roots of higher plants; a number of species of yeasts and pseudoyeasts have also been studied to some extent.

Several rather complete reviews of the literature on the subject have been given by various writers and a repetition here is unnecessary. Reference should be made especially to the papers by Lipman (*15*),[2] Goddard (*9*), Duggar and Davis (*7*), Chambers (*5*), Czapek (*6*, *pp. 192–197*), Senn (*22*), and Roberg (*19*).

Greatly increased interest was recently stimulated in the subject of nitrogen fixation by *Aspergillus* as a result of the researches reported by Schober (*20*). He studied 6 strains of *Aspergillus* and 1 species of *Citromyces* and obtained nitrogen fixation with all of these. A large number of analyses was reported of cultures grown under a considerable variation of experimental conditions. In most experiments a culture medium containing 5 percent glucose was used and the fixation was often as high as 4 mg of nitrogen per 100 cc where the growth conditions and incubation period were such as to permit most of the sugar to be utilized. Only one of the strains of *Aspergillus* used was a freshly isolated culture, the others having been grown on artificial media for varying lengths of time up to 15 years. The medium generally used was a common glucose-mineral medium containing no additions of unusual elements.

Schröder (*21*) repeated a portion of the experiments of Schober. She used 13 strains of *Aspergillus*, including several of the strains used by Schober. She also used the same medium with additions in some cases of zinc, copper, molybdenum, tungsten, and the ash of the fungus previously grown on a complete nutrient medium. Tap water was substituted for distilled water in some experiments in an effort to

[1] Received for publication Nov. 15, 1934; issued March 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 1122.

supply traces of elements which might be of importance in nitrogen fixation. Under no condition could any of the organisms use atmospheric nitrogen. Kadelbach (*14*) also worked with Schober's strains of fungi and observed that they no longer made an appreciable growth in the absence of combined nitrogen and also that their acid-producing properties had changed.

A recent paper by Roberg (*19*) reports additional data in agreement with the findings of Schröder and Kadelbach. Twenty-one strains of *Aspergillus*, including five used by Schober, were grown on media receiving additions of various metals, sea water, and other materials which might possibly supply a factor essential for fixation. The cultures were also subjected to repeated soil passage. Good growths were obtained in the flasks containing ammonium sulphate, but growth was negligible in the absence of fixed nitrogen.

Prior to the appearance of the article by Schober the authors had from time to time attempted to grow various genera of soil fungi on media very deficient in nitrogen. The growths obtained were always extremely small. Immediately following the publication of Schober's data, experiments were initiated as a direct check on his results and as an indirect check on the writers' previous observations with other species of soil fungi. In the meantime the findings of Schröder, Kadelbach, and Roberg, discussed above, appeared. The writers' experiments are reported below together with some data obtained with five species of *Actinomyces* previously reported only as a note (*1, p. 29*).

EXPERIMENTAL DATA

The cultural methods used in the experiments differed to some extent, and these variations are discussed separately for each experiment. The incubation temperature was 26° to 28° C. in all experiments. Unless otherwise stated the culture vessels used were 250-cc Erlenmeyer flasks, containing 100 cc of culture. These were fitted with cotton plugs and sterilized in the autoclave. Three media were used, the composition of which is shown in table 1.

TABLE 1.—*Composition of culture media used in experiments with fungi*

Substance	Schober's	Modified Ashby's	Burk's	Substance	Schober's	Modified Ashby's	Burk's
	Grams	*Grams*	*Grams*		*Grams*	*Grams*	*Grams*
K_2HPO_4		0.5	0.7	$CaCO_3$	5.0	10.0	
KH_2PO_4	1.0		.3	$FeCl_3$	.01	.005	0.005
NaCl		.2	.2	Glucose	50.0	10.0–50.0	10.0–50.0
$MgSO_4$	.2	.2	.2	H_2O	1,000	1,000	1,000
$CaSO_4$		.1	.1				

All analyses for total nitrogen were made by the macro Kjeldahl method, with K_2SO_4, $CuSO_4$, and H_2SO_4 in the digestion flask.

EXPERIMENT 1

In experiment 1 four strains of *Aspergillus*, used by Schober in his experiments, and obtained from the Centraalbureau Schimmelcultures, Baarn, Netherlands, were tested for ability to use atmospheric nitrogen. Three other species of *A. niger* and one of *Cladosporium*,

obtained from Charles Thom, Bureau of Plant Industry, were also included in the tests, as well as a strain of *A. niger* isolated at this laboratory. The organisms were grown on Schober's medium with and without $CaCO_3$ in cotton-plugged flasks for a period of 89 days. A barely visible growth was obtained in all flasks lacking fixed nitrogen; under the same conditions with ammonium sulphate added to the medium at the rate of 20 mg N per 100 cc very good growths were obtained with all the organisms. The analyses of these cultures, given in table 2, are in agreement with the growth observations and show no nitrogen fixation.

TABLE 2.—*Nitrogen content of cultures of Aspergillus grown on Schober's medium in the presence and absence of $CaCO_3$*

Organism	Without $CaCO_3$			With $CaCO_3$, 0.5 g per 100 cc		
	N	Average N	N fixation	N	Average N	N fixation
	Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
Check, uninoculated	0.13			0.12		
Do	.02	0.08		.14	0.13	
Aspergillus cinnamomeus (Schiemann)	.15			.24		
Do	.05	.10	0.02	.19	.22	0.09
Aspergillus fuscus (Schiemann)	.05			.17		
Do	.05	.05	−.03	.11	.14	.01
Aspergillus niger, var. *altipes* (Schiemann)	.03			.07		
Do	.07	.05	−.03	.17	.12	−.01
Aspergillus niger Tübingen (Schober)	.03			.11		
Do	.05	.05	−.03	.12	.12	−.01
Aspergillus niger no. 142 (from Thom)	.10			.15		
Do	.14	.12	.04	.10	.13	.0
Aspergillus niger no. 4202.17c (from Thom)	.05			.19		
Do	.07	.06	−.02	.24	.22	.09

NOTE.—*Aspergillus niger* no. 3528.1 (from Thom), *Aspergillus niger* (Allison), and *Cladosporium sp.* (from Thom) were not analyzed.

EXPERIMENT 2

A second experiment was carried out with six of the cultures, primarily to determine whether nitrogen fixation is dependent upon the degree of aeration of the culture. Schober's medium, containing 0.5 g $CaCO_3$ per 100 cc was used in 6 series of cultures, and Burk's medium, without $CaCO_3$, was used in the seventh series. Available iron, which has been found (*3*) to be important in the growth of *Azotobacter* and higher plants, was added to 2 series in the form of synthetic iron humate. This was prepared according to the method of Horner, Burk, and Hoover (*13*). Sufficient of this soluble form of iron was added to supply 1 part per million of iron to the culture solutions.

Two series of flasks were aerated by bubbling air through the flasks either continuously or for a half hour daily. This air was first passed through sulphuric acid to remove any traces of ammonia present. Additional aeration was obtained in another series by the use of large flasks, so as to give a large surface area.

Using the 6 cultures of *Aspergillus* in all cases, the following groups of tests were carried out: (1) Schober's medium unaerated in 2-l flasks; (2) Schober's medium aerated continuously; (3) Schober's medium aerated for a half hour daily; (4) Schober's medium unaerated in 250-cc flasks; (5) Schober's medium unaerated with iron humate; (6) Schober's medium unaerated with ammonium sulphate; and (7) Burk's medium with iron humate.

After an incubation period of 40 days no appreciable growth had taken place in any of the flasks containing nitrogen-free media. Where ammonium sulphate was supplied at the rate of 20 mg N per 100 cc the growth was very heavy with all 6 cultures of *Aspergillus*. Since the growth on free nitrogen was negligible, only two representative series were analyzed for total nitrogen. These analyses, given in table 3, agree with the growth observations and show no nitrogen fixation.

TABLE 3.—*Nitrogen content of cultures of Aspergillus grown under varying conditions of aeration*

Organism	Schober's medium with 0.5 g $CaCO_3$ per 100 cc					
	Unaerated in 2-l flasks			Aerated continuously		
	N	Average N	N fixed	N	Average N	N fixed
	Mg	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*
Checks, uninoculated	0.00			0.12		
Do	.08	0.04		.04	0.08	
Aspergillus cinnamomeus (Schiem.)	.00			.18		
Do	.07	.04	.00	.05	.12	0.04
Aspergillus fuscus (Schiem.)	.00			.07		
Do	.14	.07	.03	.00	.04	−.04
Aspergillus niger, var. *altipes* (Schiem.)	.03			.02		
Do	.08	.06	.02	.15	.09	.01
Aspergillus niger, Tübingen (Schober)	.04			.11		
Do	.00	.02	−.02	.11	.11	.03
Aspergillus niger no 142	.00			.00		
Do	.00	.00	−.04	.00	.00	−.08
Aspergillus niger no. 4202.17c	.07			.02		
Do	.03	.05	.01	.04	.03	−.05

EXPERIMENT 3

It is well known that small amounts of certain elements, especially Cu, Zn, and Mn have a marked effect on the growth of fungi. An experiment was therefore carried out to determine whether the addition of these elements in varying concentrations would enable *Aspergillus* to use atmospheric nitrogen. Both Schober's medium with 1 percent $CaCO_3$ and Burk's medium without $CaCO_3$ were used in the experiments. *Aspergillus niger* Tübingen was the test organism. Synthetic iron humate was added to a few flasks; also both chemically pure and commercial sucrose were substituted for dextrose in other flasks. The purpose in using commercial sucrose was to supply the accessory factor, "coenzyme R", which previous work (*2*) at this laboratory had shown to be essential for the respiration and growth of certain species of legume nodule bacteria and other organisms. The respiration coenzyme is commonly found in commercial sucrose in such concentrations that a 5-percent solution usually produces approximately maximum growth of legume bacteria.

The following additions were made to the two nitrogen-free media: (1) Check; (2) Cu, 0.1, 0.5, 1.0, and 5.0 p. p. m.; (3) Zn, 0.1, 0.5, 1.0, and 5.0 p. p. m.; (4) Mn, 0.1, 0.5, 1.0, and 5.0 p. p. m.; (5) Cu+Zn+Mn each in mixture 0.1, 0.5, 1.0, and 5.0 p. p. m.; (6) Cu 0.1+Zn 0.2+Mn 0.5 p. p. m. in mixture; (7) Cu 0.5, Zn 1.0, and Mn 2.5 p. p. m. in mixture; (8) synthetic iron humate 0.35 and 3.5 p. p. m. Fe; (9) Cu 0.1+Zn 0.2+Mn 0.5+Fe 0.35 p. p. m. in mixture; and

(10) sucrose c. p., 5 percent, and sucrose commercial, 5 percent, instead of dextrose. Cultures corresponding to the above groups (1) and (5) were also included with 20 mg N per 100 cc in the form of ammonium sulphate added. The Cu, Zn, and Mn were supplied in the form of sulphates.

At the end of 46 days no appreciable growth had taken place in any of the flasks except those to which 20 mg N as ammonium sulphate had been added. In the latter case the growth was heavy. Nitrogen analyses were unnecessary.

The experiments, reported above, with 9 cultures of true fungi, including 8 strains of *Aspergillus* and 1 *Cladosporium*, agree in showing no nitrogen fixation under the experimental conditions. The results in this respect are, therefore, wholly in agreement with the recently reported data of Schröder (*21*), Kadelbach (*14*), and Roberg (*19*) and in disagreement with the findings of Schober (*20*). There seems to be no obvious explanation for the anomalous findings of Schober. Apparently if the free-living soil fungi fix nitrogen, the conditions for the fixation must either be very specialized for most of these organisms or else the nitrogen-fixing power is sometimes easily lost. Since Schober reported nitrogen fixation with the seven organisms used, it seems improbable that all of the cultures would so readily lose their powers of fixation.

EXPERIMENT 4

The ability of the group of organisms known as *Actinomyces* to fix nitrogen has been studied to a comparatively limited extent. According to Waksman, as many as 40 to 60 percent of the colonies developing on common agar plates inoculated with soils are actinomyces. These organisms are not, of course, true fungi but represent a group between the fungi and bacteria, probably more nearly like the former.

In connection with the nitrogen fixation studies with the true fungi, reported above, work was also included with five representative species of actinomyces, obtained from S. A. Waksman of the New Jersey Agricultural Experiment Station. A culture of *Azotobacter vinelandii*, obtained from J. G. Lipman, also of the New Jersey station, and one of *Bacillus radiobacter*, obtained from N. R. Smith of the Bureau of Plant Industry were also included as controls. The cultures were incubated at 28° C. for 50 days on the three synthetic media given in table 1, both with and without added nitrogen, in the forms of either ammonium sulphate or asparagine. The sugar source in the case of Ashby's and also of Burk's medium was 1-percent glucose and in the case of Schober's medium 5-percent glucose. Schober's medium was also used with and without $CaCO_3$. The tests on the latter medium were carried out at a later date than the others but the experimental methods were essentially the same.

The growths of all species of actinomyces and also of *Bacillus radiobacter* were negligible on the nitrogen-free media but good in the presence of either asparagine or ammonium sulphate. *Azotobacter vinelandii*, of course, grew well both with and without added nitrogen. Although nitrogen analyses were obviously unnecessary they were made on a small portion of the cultures in order to have the absolute proof that no nitrogen fixation occurred. The analyses are given in table 4.

TABLE 4.—*Nitrogen content of cultures of actinomyces grown in the absence of fixed nitrogen*

Organism	Ashby's medium		
	N	Average N	N fixation
	Mg	*Mg*	*Mg*
Check, uninoculated	0.04 .11	0.08	
Actinomyces halstedi	.04 .09	.07	−0.01
Actinomyces flavovirens	.04 .03	.04	−.04
Actinomyces griseus	.07 .04	.06	−.02
Actinomyces californicus	.03 .00	.02	−.06
Actinomyces bobili	.03 .00	.04	−.04
Bacillus radiobacter W-1000	.00 .00	.00	−.08
Azotobacter vinelandii	3.39 3.60	3.50	3.42

The experiments with the actinomyces agree with the findings of Münter (*16*) and Waksman (*24*). Münter reported no nitrogen fixation by these organisms on neutral, acid, or alkaline media. Waksman, likewise, found no evidence of nitrogen fixation, although colonies often developed on media very deficient in nitrogen. On the other hand, Emerson (*8*) isolated 10 actinomyces (not identified) from soil and observed that all of them fixed nitrogen either in soil or solution cultures. The average fixation reported was 3.1 mg N per 50 cc of a 1-percent dextrose solution and 15.3 mg N per 100 g of sterilized soil containing no added carbohydrate. Carter and Greaves (*4*) of the Utah Agricultural Experiment Station isolated and described 10 species of actinomyces, 9 of which fixed nitrogen when incubated in pure culture in soils. The average fixation was 2.5 mg N per 100 g soil, with a maximum of 5.85 mg. No appreciable nitrogen fixation was obtained in synthetic media, the work agreeing in this respect with that reported in table 4. Later work from the same station (*10, 11, 12*) has agreed with the earlier findings that many species of soil bacteria, as well as a few fungi and actinomyces, are able to use atmospheric nitrogen when grown in sterile soils, but not when in nitrogen-free solutions. As is well known, the experimental errors connected with nitrogen-fixation studies in soil are comparatively large, and there is a need for further studies with the same organisms grown in nitrogen-free synthetic media.

DISCUSSION

A general consideration of all of the data on the subject of nitrogen fixation by fungi shows that most of the investigators, especially the more recent workers, have failed to obtain fixation with any of the species of true fungi commonly found in nature living apart from higher plants. Where fixation has been reported, the quantities fixed per unit of energy have been small, usually considerably less than 1 mg of nitrogen per gram of sugar consumed. It would seem, therefore, that nitrogen fixation is probably, at most, limited to a very few species of the free-living fungi, and even the evidence in the

case of these is not convincing. The limited data for yeasts, psuedo-yeasts, and actinomyces seem to justify a similar conclusion. It is very improbable that any of these organisms plays an important role in the maintenance of the soil nitrogen supply by means of additions from the air.

The evidence that certain endotrophic mycorrhizal fungi, particularly some of those belonging to the genus *Phoma*, can use free nitrogen is more positive, but the amount of quantitative data is limited. A portion of the evidence is based upon the fact that certain higher plants, if infected with the proper fungi, have been found to grow normally on a medium very deficient in nitrogen; in addition, some of these organisms have been reported to fix nitrogen when grown in pure culture apart from the host. It is significant that in very careful work with a large number of fungi, Duggar and Davis (*7*) obtained fixation only with *P. betae*, which, although not a mycorrhizal fungus, is possibly a closely related species. Ternetz (*23*) isolated 5 species of true mycorrhizal fungi of the genus *Phoma* and found that all could use atmospheric nitrogen, 1 culture fixing as much as 22 mg of nitrogen per gram of dextrose consumed. Rayner has considered at some length in a number of papers (especially (*18*)) the physiology and nutrition of mycorrhizal plants. Her studies (*17*) with the endophyte (*Phoma*) of *Calluna vulgaris* also indicates that this organism plays a role in nitrogen fixation. Wolff (*25*, *26*) has recently reported that the mycorrhiza from the roots of orchids can use free nitrogen but the quantities fixed were very small. Among the higher plants mycorrhizal infections of the roots are exceedingly common, but only a few of these associations have been studied with regard to nitrogen fixation. Since endotrophic mycorrhiza obtain their energy supply from the juices of the host plant, and thus indirectly from sunlight, it would seem very probable that if they possess the ability to fix nitrogen the quantity fixed may be of considerable economic importance.

SUMMARY AND CONCLUSIONS

Experiments are reported with 9 cultures of true fungi, including 8 strains of *Aspergillus* and 1 *Cladosporium*, in which an attempt was made to find experimental conditions under which these organisms can utilize atmospheric nitrogen. The results were negative in all cases. Among the cultures used were four strains of *Aspergillus*, which were obtained from the same source as Schober's and which he had previously reported as possessing this ability. The results failed to corroborate Schober's results but are in agreement with the recent findings of Schröder, Kadelbach, and Roberg.

Similar studies with five species of common soil actinomyces, grown in various media, also showed no nitrogen fixation under conditions where excellent growths occurred in the presence of combined nitrogen.

A critical consideration of all of the evidence on the subject indicates strongly that nitrogen fixation is, at most, limited to a very few species of the free-living fungi, and the data for these are certainly not conclusive. The evidence that certain mycorrhizal fungi can use atmospheric nitrogen, at least when growing in the roots of the host, is much stronger.

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SOIL-WATER MOVEMENT AS AFFECTED BY CONFINED AIR[1]

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INTRODUCTION

The effect of confined air on the movement of soil water is of sufficient importance to receive more attention than has been accorded to it. This paper records some measurements of the effect of confined air on soil-water movement obtained by the use of different devices.

A marked movement of air escaping in bubbles up through the edge of an advancing sheet of irrigation water was noted by the writer when irrigating level, square experimental checks at the New Mexico Agricultural Experiment Station some 26 years ago. A decrease in soil moisture content was consistently found in the fourth and fifth feet of soil soon after irrigation and was thought possibly to be due in part to reversion of direction of moisture movement in the subsoil below confined air.

Fauser [2] has called attention to ventilation-drainage and the effect of temperature thereon. Lewis and Neal [3] found little difference in percolation rate with a hydrostatic head of 6 inches as compared to a 1-inch head. Perhaps this was due to sealing up at the surface so as to mask the Darcy effect. Porosity was found to be important. Air (and smoke) is usually found to move upstream into tile drains flowing partly full of water and without surface vents, according to tests made locally. The suction filter and the air-pressure method of displacement of the soil solution in Parker-Burd tubes are applications of the use of confined air to facilitate percolation.

EXPERIMENTAL DATA

One type of experiment was conducted in which 250-cc burettes having a capacity for a soil column of the dimensions 3 by 40 cm were used. The soils were screened through a 2-mm sieve, and a glass-wool plug was fitted into the bottom of each burette. Soil and water were at room temperature.

EXPERIMENT 1

The first experiment was conducted to determine the effect of air on rate of (1) wetting and (2) of percolation through Chehalis loam. Three burettes were filled with soil. Tube no. 1 was left open at the base; tube no. 2 was closed; and tube no. 3 was provided with suction from the water jet equal to approximately 0.5 atmosphere. The rate of wetting and of percolation are shown in table 1.

[1] Received for publication Apr. 7, 1934; issued March 1935. Published as Technical Paper No. 220 of the Oregon Agricultural Experiment Station; contribution of the Department of Soils.

[2] FAUSER, O. PROPOSITIONS FOR THE INTERNATIONAL DEVELPOMENT OF THE ORGANIZATION OF DRAINAGE EXPERIMENTS. Internatl. Soc. Soil Sci. Proc. v. 1, pp. 21-39. 1930.

[3] LEWIS, M. R., and NEAL, E. H. RATE OF INFILTRATION OF WATER INTO SOIL. Agr. Engin. 9: 147-148, illus. 1928.

It will be seen that it took about twice as long to wet down the tube with confined air at its base, and approximately one-thirteenth as long where suction was applied. Sealing up or rearrangement of soil particles appears to have largely inhibited the effect of suction on rate of percolation.

TABLE 1.—*Rate of wetting and percolation through Chehalis loam columns (in burettes) 3 cm wide and 40 cm deep*

Experiment and duration	Distance wet down and amount of percolate through soil in burette no.—		
	1; open	2; closed	3; suction applied (0.5 atmosphere)
Experiment 1a, depth of wetting:	*Cm*	*Cm*	*Cm*
45 seconds	14	7.5	[1] 40
5 minutes	29	18	
10 minutes	[1] 40	22	
Experiment 1b, amount of percolate:	*Cc*		*Cc*
First 10 minutes	75		75
Second 10 minutes	75		75
Third 10 minutes	75		70

[1] Wet through.

The experiment on rate of wetting was repeated with Willamette silty clay loam. The results are shown in table 2. The rate of wetting was slower, and the effect of suction or of confined air was less marked with this heavy-textured soil.

EXPERIMENT 2

To determine the rate of percolation through soil columns of different lengths, columns of Willamette silty clay loam 10, 20, and 40 cm in height were arranged in burettes as before and connected with suction. A second 40-cm column was provided as a check. The rates of percolation are shown in table 3.

TABLE 2.—*Rate of wetting through Willamette silty clay loam columns (in burettes) 3 cm wide and 40 cm deep*

Time (minutes)	Distance wet down in burette no.—			Time (minutes)	Distance wet down in burette no.—		
	1; open	2; closed	3; suction applied (0.5 atmosphere)		1; open	2; closed	3; suction applied (0.5 atmosphere)
	Cm	*Cm*	*Cm*		*Cm*	*Cm*	*Cm*
10	6.0	4.5	18	60	13.5	9.5	35
20	7.5	6.5	24	80	15.0	11.0	[1] 40
40	10.5	8.0	29	100	24.0	16.0	

[1] Wet through.

TABLE 3.—*Percolation through soil columns of different depths (in burettes) containing Willamette silty clay loam*

Duration (hours)	Amount of percolate through soil column of indicated depth—			
	40 cm, no suction	40 cm, with suction	20 cm, with suction	10 cm, with suction
	Cc	*Cc*	*Cc*	*Cc*
1	6	8	----------	29
2	4	7	----------	21
3	5	8	12	----------
4	6	8	14	----------

Apparently increasing the length of a soil column to 40 cm nearly overcomes the suction head with silty clay-loam soil. These results give meager support to the supposition that suction aids tile drainage, for it appears that even within short distances it tends to rearrange soil particles in such a way as to decrease percolation with time.

EXPERIMENT 3

A different method of studying the relation of depth of soil column to percolation of water under different air pressures was used. For this work Parker-Burd soil-solution-displacement tubes 8 by 40 cm

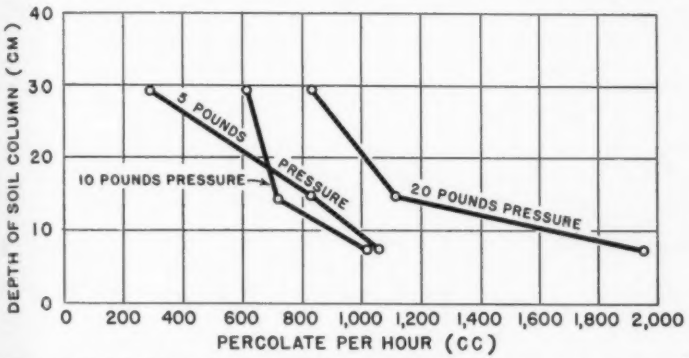

FIGURE 1.—Rate of percolation through fine sandy loam as determined by the use of Parker-Burd soil-solution-displacement tubes 8 by 40 cm in dimensions, with different air pressures, in pounds.

were employed. Fine sandy loam was used at different depths and under different heads as shown in table 4. The data are presented graphically in figure 1. With a greater height of soil column the rate of percolation may in time become fairly constant or give a fairly straight line for the upper part of the curve if extended.

TABLE 4.—*Effect of depth of soil column on rate of percolation through fine sandy loam in Parker-Burd soil-solution-displacement tubes*

Pressure and percolation period	Percolate for tube no.[1] —		
	1; 7.3 cm soil depth	2; 14.5 cm soil depth	3; 29.4 cm soil depth
No air pressure:	*Cc*	*Cc*	*Cc*
Percolate during first 30 minutes	24	0	0
Percolate during second 30 minutes	53	0	0
5 pounds air pressure per square inch:			
Percolate during first 30 minutes	629	455	85
Percolate during second 30 minutes	429	381	212
10 pounds air pressure per square inch:			
Percolate during first 30 minutes	590	407	367
Percolate during second 30 minutes	426	318	235
20 pounds air pressure per square inch:			
Percolate during first 30 minutes	995	612	463
Percolate during second 30 minutes	964	496	372
Total water added at top of column	4,000	3,500	3,035

[1] The weight of air-dry soil in tube no. 1 was 500 g, in no. 2, 1,000 g, and in no. 3, 2,000 g; the time required for percolation to start was 18, 61, and 80 minutes, respectively.

A pressure of 1 pound per square inch is equivalent to a hydrostatic head of 2.31 feet. The water layer perhaps gives a more uniform application of pressure than would occur without it. An additional average head of approximately 5 cm was common to all tubes in these tests because of the water added above the soil.

In nearly all cases there was a decrease in rate of percolation with time which may have come about with rearrangement of soil particles and dispersion and swelling of colloids following displacement of solutes originally present under pressure applied. The effect of the 5-pound pressure appears to have been largely masked by the 29-cm soil column.

The effect of pressure and length of soil column on rate of percolation through soil-solution-displacement tubes was also studied with silty clay loam. The results are shown in table 5.

TABLE 5.—*Effect of pressure and depth of soil column on rate of percolation through Willamette silty clay loam in soil-solution-displacement tubes*

Percolation period	Data for tube no.[1] —							
	1; 7.5 cm soil depth		2; 7.5 cm soil depth		3; 14.5 cm soil depth		4; 21.6 cm soil depth	
	Air pressure	Percolate	Air pressure	Percolate	Air pressure	Percolate	Air pressure	Percolate
	Pounds	*Cc*	*Pounds*	*Cc*	*Pounds*	*Cc*	*Pounds*	*Cc*
First 30 minutes	0	2	0	2	0	1	0	0
Second 30 minutes	0	4	5	38	5	15	5	[2] 1
Third 30 minutes	0	3	5	37	5	16	5	10
Fourth 30 minutes	0	4	10	62	10	34	10	18
Fifth 30 minutes	0	3	10	51	10	30	10	17
Sixth 30 minutes	0	3	20	78	20	61	20	37

[1] The weight of soil in tubes nos. 1 and 2 was 500 g, that in no. 3, 1,000, and that in no. 4, 1,500; the respective time required to wet the soil through was <24 hours for the first 3 tubes and 24 hours for no. 4.
[2] Delayed in wetting down.

Pressure increased percolation 9 to 26 times through the 7.5-cm column, 4 to 20 times through the 14.5-cm column, and 3 to 12 times through the 21.6-cm column. When these data are plotted, the discharge appears to be proportional to some fractional power of the hydraulic gradient. The rate of flow through fine sandy loam was of the order of 10 times the quantity passing through silty clay loam under similar conditions.

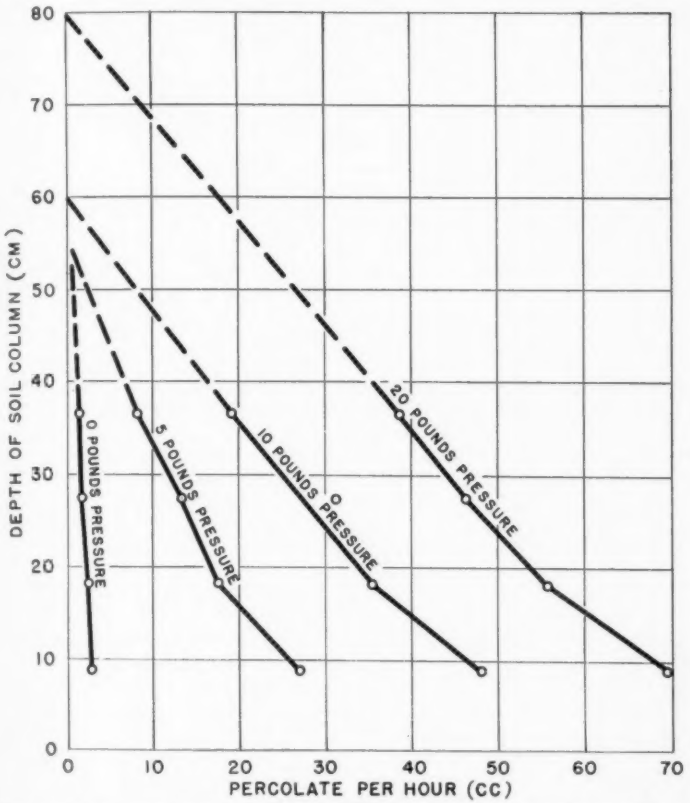

FIGURE 2.—Rate of percolation through columns of Willamette silty clay loam of different depths, with different air pressures.

Larger amounts of soil and longer soil columns were used in another trial similar to that just described. The results secured are shown in table 6.

The effect of pressure on the amount of percolation appears to decrease somewhat with the longer columns, yet is not entirely masked thereby. These results are shown graphically in figure 2.

EXPERIMENT 4

The effect of texture and pressure upon movement of water through soil columns in soil-solution-displacement tubes was studied as described above. The results are shown in table 7.

TABLE 6.—*Effect of pressure and depth of soil column on rate of percolation through Willamette silty clay loam*

Pressure and percolation period	Percolate per hour for tube no.[1] —			
	1; 9.1 cm soil depth	2; 18.2 cm soil depth	3; 27.4 cm soil depth	4; 36.4 cm soil depth
No pressure:	*Cc*	*Cc*	*Cc*	*Cc*
5-hour test	3.0	2.6	2.4	2.4
18-hour test	2.8	2.3	2.2	1.9
16-hour test	2.3	1.9	1.6	1.4
5-pound pressure:				
First 30 minutes	29.0	19.0	14.0	9.0
Second 30 minutes	26	17	13	8
10-pound pressure:				
First 30 minutes	54.0	40.0	35.0	22.0
Second 30 minutes	42.0	31	27	17
20-pound pressure:				
First 30 minutes	73.0	62.0	52.0	43.0
	66	50	41	34

[1] The weight of soil in tube no. 1 was 600 g; in no. 2, 1,200 g; in no. 3, 1,800 g; in no. 4, 2,400 g.

TABLE 7.—*Effect of texture on movement of water through columns of three different types of soil, in soil-solution-displacement tubes, under different pressures, 1,000 g of air-dry soil being used in each tube*

Pressure and percolation period	Percolate through—					
	Sandy loam in tube no. —		Loam in tube no. —		Silty clay loam in tube no. —	
	1; 14.5 cm soil depth	2; 14.6 cm soil depth	3; 14.5 cm soil depth	4; 14.7 cm soil depth	5; 15.1 cm soil depth	6; 15.3 cm soil depth
No pressure:	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*	*Cc*
First 30 minutes	44	30	12	14	1	1
Second 30 minutes	28	25	11	12	2	3
5 pounds pressure:						
First 30 minutes	430	320	75	77	19	20
Second 30 minutes	331	245	54	53	23	27
10 pounds pressure:						
First 30 minutes	458	341	83	83	35	37
Second 30 minutes	342	248	79	78	34	36
20 pounds pressure:						
First 30 minutes	542	400	174	180	66	68
Second 30 minutes	382	248	117	121	57	59
Total water added	4,000	3,500	1,750	1,750	1,500	1,500

Table 7 shows that texture has a marked effect on the movement of water. The rate of flow of water through soil columns in these trials decreased with time.

EXPERIMENT 5

To learn what pressure could be developed, a 15-cm iron pipe 2 m tall to which piezometers could be attached at 30-cm vertical intervals was filled with sandy loam, and a head of 3 cm maintained thereon. A hydrostatic head of 30 cm was developed in 12 hours on the bottom

gage. There is a tendency for confined air to break through to the surface or to lift the saturated layer of soil near the surface in this type of tube experiment.

EXPERIMENT 6

An experiment was made to determine whether confined air affects the movement of capillary soil moisture. Four glass tubes each 3 by 45 cm were closed by fastening small pieces of linen over one end and were then filled with screened Willamette silty clay loam. They were supported in a pan containing water to a depth of 2 cm for 1 hour, and during that time they wet up 7 to 8 cm. The water was then removed and they were allowed to drain overnight. The following morning the position of the moist capillary fringe was marked on the glass with a blue wax pencil. The tubes were then divided into pairs, and one member of each pair was used as a check. The other tubes, one at a time, were connected to an air-pressure line by means of an airtight tube, so that air pressure of approximately 1 pound per square inch could be maintained on the base of the moist soil columns. The results of the tests thus made are reported in table 8.

TABLE 8.—*Effect of confined air upon the capillary movement of soil moisture in Willamette silty clay loam, with different pressures*

Time (hours)	Height of rise of capillary moisture (mm per hour) in tube no.—			
	1; no pressure	2; no pressure	3; 1 pound per square inch	4; 1 pound per square inch
First	2	2	5	5
Second	2	2	5	5
Third	2	1.5	5	5
Total	6	5.5	15	15

Air pressure and movement definitely increased the extent of capillary rise of moisture in the soil column. The moisture advanced about 2½ times as far when air pressure was applied. There was a definite movement of air up through the soil columns to which it was applied. Increasing the air pressure tended to cause a break or channeling in the upper or dry part of the soil column.

EXPERIMENT 7

Movement of water through soils in the natural field structure is affected by air pressure.

An effort was made to determine whether the rate of percolation in time would be little affected where soil was used in the natural field structure to lessen or avoid rearrangement of soil particles. A dozen brass percolation tubes 5 by 25 cm in size were carefully forced into slightly moist land so as to secure columns of soil in the natural field structure. Six tubes were filled with Chehalis loam and six with Willamette silty clay loam. Soil was cut out of the inverted lower end of each column to a depth of 3 cm to permit water to be kept over the surface of each. One pair of tubes containing each soil type

was left open at the lower end. Companion sets were corked airtight and the remaining pairs were connected through traps to air suction as in experiment 1.

When percolation began in all except the sealed tubes of each type of soil, these were emptied and examined. The water in the meantime had wet down approximately 11 cm or half-way through. Percolation rates are shown in table 9.

The air suction greatly increased the rate of percolation. In the loam the rate of percolation remained fairly constant after a moderate decrease during the first few intervals, but in the heavy-textured soil it continued to decrease. Perhaps leaching resulted in progressive dispersion and swelling of ultraclay particles. Similar results previously had been secured in these laboratories, where rate of percolation through heavy soil continued to decrease during a 390-hour period.[4] Scratching up the surface before the final two periods of test on the Willamette silty clay loam increased the rate of percolation, which indicates that some sealing occurs from puddling at the surface.

TABLE 9.—*Effect of time and aeration on percolation through Chehalis loam and Willamette silty clay loam soil monoliths in brass percolation tubes*

CHEHALIS LOAM

Percolate	Percolate through tube no.—			
	1; open	2; open	5; with suction	6; with suction
First day:	*Cc*	*Cc*	*Cc*	*Cc*
First 15 minutes	18	15	45	43
Second 15 minutes	16	14	46	41
Third 15 minutes	14	11	41	38
Fourth 15 minutes	14	11	43	39
Fifth 15 minutes	14	12	40	35
Sixth 15 minutes	14	12	34	30
Second day:				
First 15 minutes	14	12	42	30
Second 15 minutes	12	11	35	28
Third 15 minutes	12	10	36	29
Fourth 15 minutes	11	10	40	30
	Minutes	*Minutes*	*Minutes*	*Minutes*
Time required for penetration	45	50	4	5

WILLAMETTE SILTY CLAY LOAM

First day:	*Cc*	*Cc*	*Cc*	*Cc*
First 2 hours	9	9	25	24
Second 2 hours	8	7	24	27
Second day:				
Third 2 hours	6	6	24	20
Fourth 2 hours	5	5	20	18
Fifth 2 hours	3	3	19	17
Sixth 2 hours	2½	2	17	16
Surface one-half inch, cultivated:				
Seventh 2 hours	5	5	20	20
Eighth 2 hours	3	3	20	18
	Minutes	*Minutes*	*Minutes*	*Minutes*
Time required for penetration	720	780	97	92

[4] POWERS, W. L. A STUDY OF THE COLLOIDAL FRACTION OF CERTAIN SOILS HAVING RESTRICTED DRAINAGE. Soil Sci. 23: 487–491, illus. 1927.

DISCUSSION

In the light of these experiments, any value of suction in a tile drain would seem to be limited to short distances. Confined air may impede penetration of irrigation water less with the furrow method of irrigation than with flooding. Movement of soil water may be expected to cause movement of soil air. Whether there is pressure or not, the rate of percolation slows up at least for a time, perhaps due to rearrangement of soil particles following removal of solutes and dispersion of soil crumbs. Swelling of colloids may also check percolation for a considerable time. After these factors are stabilized and with long soil columns the relation between percolate and time may become fairly constant for a given height of soil column or show a relation approaching a straight line. This condition appears to develop more promptly in loam than in silty clay loam. Rate of flow should increase with increased pressure yet the flow per pound pressure would be less. The increased rate of capillary movement in the presence of partly confined air may be partly due to transportation in vapor form.

Control of temperature, preventilation of soil, and saturation of soil water used with air would also seem desirable. Previous leaching of the soil used might also reduce interference of substances in solution. The presence of active decaying organic matter and plant root activity under field conditions will be modifying factors. Barometric pressure should also be observed. A change in soil air pressure will disturb the equilibrium between the gas-liquid and liquid-solid phases of the system and some loss in the gaseous phase may occur. Viscosity of the air or gaseous phase may be a factor. The trials herein reported were with nearly pure water. Sedimentation on and dispersion at the soil surface retard infiltration of water into soil.

SUMMARY AND CONCLUSIONS

The rate of wetting sandy loam was about twice as fast in an open tube as in one closed at the base. There is a slowing up in the rate of wetting as the air becomes compacted toward the base of a closed tube. Sufficient suction to produce the equivalent of approximately 0.5 atmosphere caused the rate of advance of moisture down through the tube to be many times as fast as in tubes dependent upon gravity alone.

The rate of percolation did not seem to be increased appreciably by suction after percolation began. It appears that the friction largely overcomes suction force even in a fairly short column of soil. Where silty clay loam is substituted, a similar relative rate of wetting is observed. The rate of percolation, especially in heavy-textured soils, decreases with time, perhaps as a result of displacement of the soil solution, swelling of colloids, and rearrangement of soil particles under pressure in soil-solution-displacement tubes.

Air pressure definitely increased vertical movement of capillary soil moisture.

Any value of suction in tile drains would seem to be limited to short intervals. Furrow irrigation would tend to confine soil air less than flooding and therefore may favor penetration of irrigation water.

Dispersion at the soil surface retards infiltration of water.

TRANSMISSIBILITY BY APHIDS OF THE TOBACCO MOSAIC VIRUS FROM DIFFERENT HOSTS[1]

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INTRODUCTION

It is now fairly well established that heavy infections with ordinary tobacco mosaic (tobacco virus 1) (*8*)[2] in tobacco fields are not attributable to dissemination of the virus by aphids. Not only has the writer presented evidence that aphids do not transmit the tobacco mosaic virus from tobacco to tobacco (*4*, *5*), but Johnson and Ogden (*10*), Valleau and Johnson (*11*), and others have shown that extensive attacks may be adequately accounted for on the basis of other modes of infection and spread.

The common occurrence of a limited amount of sporadic infection in tobacco fields under conditions apparently precluding infection from the usual sources is not so easily explained. The possibility that such sporadic infection arises from aphid transmission of the tobacco mosaic virus from certain other hosts appears to deserve consideration, especially since these insects have been shown to transmit this virus from tomato (*5*). A more thorough survey has therefore been made of various hosts of the tobacco mosaic virus with respect to possible aphid transmission. Particular attention has been directed to certain perennial weeds in which the virus has been found to overwinter (*1*, *2*), and to various nonsolanaceous plant species recently shown by Grant (*3*) to be susceptible to the virus. In this connection it is desirable not only to determine whether aphids can transmit the virus from the host in question but also to consider the proportion of insects acting as vectors, the number of such hosts in the vicinity of tobacco fields, the degree of aphid infestation, and so on. Detailed field surveys, however, have not been included in this investigation.

MATERIALS AND METHODS

The present study is essentially a continuation of earlier investigations (*4*, *5*), and the methods employed were, in general, the same as those previously reported. Some minor improvements in technic have been introduced as the result of experience gained in the course of the work. These include the use of newly infected plants as sources of virus and the transference of aphids from them after quite short feeding periods (usually about 1 day). The experiments have been continued over a period of several years (1930–34), since only certain times of the year, particularly the spring and fall, were found favorable for aphid transmission studies in the greenhouse under Wisconsin con-

[1] Received for publication Oct. 4, 1934; issued March 1935. Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to literature Cited, p. 141.

ditions. The remarkable constancy of results obtained from season to season has, however, been a striking feature of the investigation.

Three species of aphids were tested for their ability to transmit the tobacco mosaic virus from the different hosts, namely, *Myzus pseudosolani* (Theob.), *M. persicae* (Sulz.), and *Macrosiphum solanifolii* (Ashm.). These were for the most part derived from colonies used in the earlier work. In 1933, however, the colony of *Myzus pseudosolani* was lost as a result of hot weather, and a new colony was obtained from Presque Isle, Maine.[3] This strain appeared similar to the original one as regards virus transmission, although it multiplied less readily in the greenhouse. As a control, the aphids were tested also for their ability to transmit the cucumber mosaic virus (cucumber virus 1) (*8*) from the various hosts under similar conditions.

Perennial solanaceous weed hosts tested were *Physalis longifolia* Nutt., *P. heterophylla* Nees, and *Solanum carolinense* L. *Solanum nigrum* L. was tested further, since some evidence of transmission of the tobacco mosaic virus from this species by *Myzus pseudosolani* was obtained earlier (*5*). The garden huckleberry, *S. nigrum* var. *guineense* L. was also included. Other solanaceous genera were represented by *Nicandra physalodes* (L.) Pers., *Capsicum annuum* L., *Petunia violacea* Lindl., and the currant tomato, *Lycopersicum pimpinellifolium* Dunal. Hosts in other families were selected as follows: *Cynoglossum amabile* Stapf and Drummond (Boraginaceae); *Emilia sagittata* (Vahl) DC. and *Zinnia elegans* Jacq. (Compositae); *Fagopyrum esculentum* Moench. (Polygonaceae); *Martynia louisiana* Mill. (Martyniaceae); *Phacelia whitlavia* Gray (Hydrophyllaceae); and *Spinacia oleracea* L. (Chenopodiaceae). Comparative tests were made at intervals from tobacco (*Nicotiana tabacum* L. var. Connecticut Havana) and from tomato (*L. esculentum* Mill.).

In previous trials (*5*) occasional tobacco mosaic infections had been noted in certain transfers, which could not be definitely attributed to aphid transmission, partly because of their rarity and partly because a very few infections developed also on the control plants. In the present work, therefore, efforts were made to reduce still further the chances of accidental contamination and at the same time to increase, if possible, the chances of aphid transmission of the tobacco mosaic virus if this did occasionally take place from certain hosts. For the second purpose, aphids were transferred in very large numbers—300 or more—from the mosaic-diseased host to each of a total of 50 tobacco plants. This involved a number of trials at different times. The aphids were transferred by the "leaf method" previously described (*4*, *7*), and, as controls, a like number of plants were treated with diseased leaves free from aphids, as in previous experiments. In trials with the cucumber mosaic virus, transfers were made similarly to a total of 10 tobacco plants, though in these tests fewer aphids were usually used.

In addition to the regular precautions observed against accidental contamination with the tobacco mosaic virus, including careful choice of soil, steam sterilization of pots, washing of hands with soap and water, and so on, more rigorous measures were introduced to avoid contact of the test plants with any virus-bearing materials throughout the trials. Thus, particular care was taken to prevent contact of the

[3] This colony was kindly supplied by Dr. E. S. Schultz.

plants with one another or with the walls of the cages in which they were confined, and to reduce handling of the plants to a minimum. As a result of these precautions, no tobacco mosaic infection was observed on any control plant throughout the investigation.

EXPERIMENTAL RESULTS

The results obtained in tests of the transmissibility of the tobacco and cucumber mosaic viruses from the different hosts by the three species of aphids are summarized in table 1. As all control plants remained free from infection throughout, these have been omitted from the table.

TABLE 1.—*Transmission of the ordinary tobacco mosaic virus and the cucumber mosaic virus to tobacco from various hosts by three species of aphids*

[In tests with the tobacco mosaic virus, 300 or more aphids were transferred to each of 50 tobacco plants]

Mosaic host (source of virus)	Plants infected with tobacco mosaic virus by aphids indicated						Plants infected with cucumber mosaic virus by aphids indicated					
	Myzus pseudo-solani		*Macro-siphum solanifolii*		*Myzus persicae*		*Myzus pseudo-solani*		*Macro-siphum solanifolii*		*Myzus persicae*	
	Number	Per-cent	Number	Per-cent	Number	Per-cent	Number[1]	Per-cent	Number[1]	Per-cent	Number[1]	Per-cent
Nicotiana tabacum	1	2	0	0	1	2	6	60	6	60	7	70
Lycopersicum esculentum	39	78	30	60	12	24	7	70	6	60	9	90
L. pimpinellifolium	42	84	37	74	19	38	7	70	5	50	7	70
Solanum nigrum	9	18	5	10	0	0	9	90	7	70	8	80
S. nigrum var. *guineense*	8	16	1	2	2	4	2	20	4	40	8	80
Nicandra physalodes			7	14	5	10					10	100
S. carolinense	0	0	0	0	0	0	10	100	5	50	9	90
Physalis longifolia	0	0	0	0	0	0	5	50			10	100
P. heterophylla	0	0	0	0	0	0	9	90			7	70
Capsicum annuum	0	0	0	0	0	0	10	100	10	100	9	90
Petunia violacea	0	0	0	0	0	0	10	100	10	100	10	100
Martynia louisiana	10	20			4	8					6	60
Spinacia oleracea	5	10	1	2	7	14			9	90	10	100
Fagopyrum esculentum	4	8	2	4	0	0	6	60	7	70	9	90
Emilia sagittata					3	6					6	60
Zinnia elegans	0	0	0	0	0	0	7	70	6	60	10	100
Cynoglossum amabile	0	0	0	0	0	0	7	70	5	50	8	80
Phacelia whitlavia	0	0	0	0	0	0					7	70

[1] Figures in this column represent number of plants infected of 10 tested.

The cucumber mosaic virus was found to be transmissible from all hosts tested and by each species of aphid, usually in relatively high percentages of infection (50–100 percent). *Solanum nigrum* var. *guineense* was exceptional in yielding only a low proportion of infection with this virus in trials with *Myzus pseudosolani* and *Macrosiphum solanifolii*. These limited tests suggested that conditions were, in general, favorable for aphid transmission and that transmission of the tobacco mosaic virus might also be expected to occur if this virus was, indeed, transmissible from the various hosts by aphids. In this connection, several hitherto unrecorded hosts of the cucumber mosaic virus have been determined, namely: *Lycopersicum pimpinellifolium*, *S. nigrum* var. *guineense*, *Nicandra physalodes*, *Physalis longifolia*, *P. heterophylla*, *Cynoglossum amabile*, *Zinnia elegans*, and *Phacelia whitlavia*. The last two, however, have recently been reported as susceptible to the closely related southern celery mosaic virus (*12*).

Transmission of the tobacco mosaic virus was obtained, as before, from tomato, *Lycopersicum esculentum* var. Marglobe, by *Myzus*

pseudosolani and *Macrosiphum solanifolii*, and in about the same amount (78 and 60 percent infection, respectively, as compared with 85 and 48 percent in earlier trials (*5*)). More definite evidence was secured in these tests that *Myzus persicae* may to some extent also transmit the tobacco mosaic virus from this host. In transfers of this aphid from tomato, 24 percent infection resulted, although in previous trials only 2 plants in 65 had become diseased (*5*). This higher percentage of infection is probably attributable partly to the greater numbers of aphids transferred to each plant and partly perhaps to improved technic. Somewhat higher percentages of infection were obtained with each aphid in corresponding trials with *L. pimpinellifolium* (84, 74, and 38 percent, respectively). That transmission should take place from this host was not surprising on account of its close relationship to *L. esculentum*. These were the only two species, however, from which transmission of the tobacco mosaic virus occurred with any regularity. No transmission was obtained from tobacco, with two possible exceptions, a single plant becoming infected in trials with *M. pseudosolani* and with *M. persicae*, respectively. It will be noted, however, that occasional infections developed in trials with certain other hosts. Thus, *Solanum nigrum* yielded 18 percent infection with *M. pseudosolani* as compared with 12 percent obtained in previous trials (*5*), and 10 percent with *Macrosiphum solanifolii*. Occasional infections, varying from 2 to 20 percent, developed in trials from certain other hosts, namely: *S. nigrum* var. *guineense*, *Martynia louisiana*, *Fagopyrum esculentum*, *Spinacia oleracea*, *Emilia sagittata*, and *Nicandra physalodes*, while from the remaining 8 hosts, including the 3 perennial solanaceous weeds, no transmission whatsoever took place. In nearly all instances where infection occurred, the highest percentages were due to *Myzus pseudosolani* and the lowest to *M. persicae*. This same relation held in tests of the two species of tomato. Spinach was an exception in that the greatest amount of infection from this host occurred in trials with *M. persicae*. These occasional infections are presumably to be attributed to a rare ability of the aphids, particularly *M. pseudosolani*, to transmit the tobacco mosaic virus from the hosts in question. The possibility has not been overlooked that they might be due to the occasional presence of some other insect acting as vector. However, there is as yet no convincing evidence that such common greenhouse insects as thrips or white fly are able to transmit the tobacco mosaic virus, and the limited tests performed by the writer with these insects have given negative results. Moreover, the fact that a significant amount of transmission was obtainable from tomato, particularly with one species of aphid, supports the contention that the less frequent infections occurring in trials with other hosts were also the result of aphid transmission.

It seemed difficult, nevertheless, to account for these occasional transmissions from certain hosts. There appeared to be no relation between the amount of infection obtained and the favorableness of the host as a food plant for the aphids. For example, *Cynoglossum amabile* was apparently very well liked by both *Myzus pseudosolani* and *Macrosiphum solanifolii*, but no transmission of the tobacco mosaic virus took place from this host. Nor was any relation apparent between the amount of transmission of the tobacco mosaic virus and of the cucumber mosaic virus from any host. The fact that comparatively high percentages of infection with tobacco mosaic were

obtained from tomato, particularly with *Myzus pseudosolani*, did not necessarily imply that a large proportion of aphids were actually serving as vectors, although this might at first appear to be so, on account of the great number of insects used in each transfer. Experiments were planned, therefore, to determine more closely the relative number of aphids bringing about infection with the tobacco mosaic virus from tomato. For this purpose, a hybrid plant, *Nicotiana tabacum* × *N. glutinosa* was used. This plant develops local necrotic lesions on the leaves at points of infection with tobacco mosaic (*9*), as does *N. glutinosa*, or necrotic streaks if the virus is introduced directly into the veins. *Lycopersicum pimpinellifolium* served as the source of the virus, since this species had yielded the greatest amount of transmission in previous trials. Aphids were transferred by the usual method from recently infected *L. pimpinellifolium* to young hybrid plants; the approximate number of aphids was estimated at the time of transference, and the number of local lesions of tobacco mosaic resulting on the hybrid was determined later. Although the aphids appeared to feed fairly readily on this host, surprisingly few

TABLE 2.—*Number of local lesions of tobacco mosaic produced on hybrid Nicotiana tabacum* × *N. glutinosa by three species of aphids from mosaic-diseased Lycopersicum pimpinellifolium*

Experiment no.	*Myzus pseudosolani*		*Macrosiphum solanifolii*		*Myzus persicae*	
	Aphids	Lesions	Aphids	Lesions	Aphids	Lesions
	Number [1]	*Number*	*Number* [1]	*Number*	*Number* [1]	*Number*
1	1,060	8	760	6	3,420	5
2	1,100	8	1,090	9	3,110	3
3	680	6	1,925	12	3,220	4
Total	2,840	22	3,775	27	9,750	12
Approximate proportion of lesions to aphids	1:129		1:140		1:812	

[1] Approximate.

lesions developed (table 2), even with *M. pseudosolani*. Thus, from a total of 2,840 individuals of *M. pseudosolani* transferred to the hybrid, only 22 lesions were produced. As these lesions occurred in isolated positions on the leaves it was assumed that each was caused by a different aphid. Hence it was estimated that, on an average, about 1 individual in 129 actually transmitted the tobacco mosaic virus from *L. pimpinellifolium*. The numerous feeding marks, characteristic of this aphid, distributed over the leaves of the hybrid afforded visual demonstration that many individuals had fed on the plants without causing infection. Similar estimates made with the other two species of aphids indicated that transmission was effected by about 1 aphid in 140 with *Macrosiphum solanifolii* and only about 1 aphid in 800 or more with *Myzus persicae*. All control plants remained free from lesions. Other trials with *M. persicae*, made during the winter when conditions were unfavorable for transmission work, yielded infection from 1 aphid in about 1,300. Although there is some reason to believe that the hybrid is somewhat less susceptible to infection with tobacco mosaic than *N. tabacum* (*9*), it is evident that,

even from tomato, aphids do not transmit the tobacco mosaic virus nearly as readily as might be supposed from the earlier results.

Significant comparisons may be made of the relative transmissibility by aphids of this and certain other mosaic viruses. Earlier studies of the transmission of the cucumber mosaic virus by *Myzus persicae* (7) indicated that, when transferred from tobacco to tobacco, about 1 aphid in 15 or 16 caused infection, and when transferred from tobacco to cucumber, about 1 aphid in 7. Comparative tests were now made with a crucifer mosaic virus, which has been found to produce conspicuous necrotic local lesions on tobacco when transmitted by aphids or by artificial inoculation.[4] Single aphids (*M. persicae*) were transferred from cabbage seedlings infected with this virus to each of a number of young tobacco plants and the number of lesions developing was determined about a week later. A total of 60 aphids thus transferred caused 14 lesions, representing infection from 1 aphid in 4 or 5. Preliminary trials with the sugar-beet mosaic virus, which produces chlorotic or faintly necrotic local lesions on tobacco when transferred by *M. persicae* (6) indicated that, on an average, approximately 1 aphid in 4 transmitted this virus from sugar beet. These viruses are obviously much more readily transmitted by aphids than is the tobacco mosaic virus from tomato, and it is evident that the proportion of viruliferous insects in transfers from some of the other hosts of tobacco mosaic tested must be exceedingly low. The number and variety of host species studied in this connection lend weight to the conclusion that the tobacco mosaic virus is not well adapted to transmission by aphids, and that it is transmissible only rarely, or with difficulty, by these insects.

DISCUSSION

The evidence presented in this and in preceding papers (*4*, *5*) shows that the possibility of introduction of the ordinary tobacco mosaic virus into tobacco fields from wild or other hosts through the agency of aphids is a factor of minor consideration in relation to the origin of infection or to subsequent dissemination. The data do not, of course, justify the conclusion that infection of tobacco may not occasionally originate from insect transmission from some such commonly grown host as tomato. It remains to be shown, however, that this or other hosts from which transmission may occur support aphids in sufficient numbers to account for the regular occurrence of sporadic infection observed in tobacco fields, particularly when one takes into consideration the low proportion of aphids that become viruliferous. As has already been pointed out, the highly contagious and resistant nature of the tobacco mosaic virus allows its introduction and spread in the field by other means and to a sufficient extent to account for infection up to 100 percent of the plants.

Although this series of investigations on aphid transmission of plant viruses has been conducted with a view to the bearing of results on disease control, a clearer understanding of the relation between virus and aphid has been sought at the same time. The factors influencing the transmissibility of the tobacco mosaic virus by aphids

[4] HOGGAN, I. A., and JOHNSON, J. A VIRUS OF CRUCIFERS AND OTHER HOSTS. Phytopathology (in press).

have been particularly obscure. It is now fairly evident that transmission of this virus is not determined by such factors as the concentration of the virus in the host or the suitability of the host as a food plant for the aphid. Nor has any further evidence become available that the distribution of the virus in the host may be a determining factor. It is difficult also to account for the fact that *Myzus persicae*, usually regarded as the most efficient transmitter of viruses of the three species of aphids tested, is distinctly the least efficient in the transmission of the tobacco mosaic virus. The varying proportions of aphids able to act as vectors, according to the specific virus tested and the host plants used, point to the existence of a complex relationship between the virus, the host tissues, and the aphid.

SUMMARY

In repeated tests of the ability of three species of aphids in large numbers to transmit the ordinary tobacco mosaic virus from various solanaceous and other hosts, transmission was obtained with regularity only from tomato (*Lycopersicum esculentum* and *L. pimpinellifolium*). Occasional infections developed in trials with 8 other hosts; from the remaining 8 hosts tested no evidence of transmission was obtained. In comparative trials with the cucumber mosaic virus, relatively high percentages of infection were obtained from all hosts tested, with the exception of *Solanum nigrum* var. *guineense*.

In general, the greatest amount of transmission of the tobacco mosaic virus from any host was effected by *Myzus pseudosolani;* less infection was obtained with *Macrosiphum solanifolii* and least with *Myzus persicae*.

By transferring known numbers of aphids from tobacco-mosaic-diseased *Lycopersicum pimpinellifolium* to plants of the hybrid *Nicotiana tabacum* × *N. glutinosa* and counting the number of local lesions of tobacco mosaic produced, it was estimated that, with *Myzus pseudosolani*, about 1 aphid in 129 caused infection; with *Macrosiphum solanifolii* about 1 aphid in 140; and with *Myzus persicae* about 1 aphid in 800 or more. Comparative tests indicated that, in the transmission of a crucifer mosaic virus by *M. persicae* to tobacco, 1 aphid in 4 or 5 acted as vector, and, in transmission of the sugar-beet mosaic virus to tobacco by the same aphid, approximately 1 aphid in 4.

It is concluded that the more common aphids probably do not transmit the ordinary tobacco mosaic virus from certain hosts, and only rarely or with great difficulty from other hosts. It not only seems unlikely that any appreciable amount of dissemination of tobacco mosaic may be brought about by aphids, except perhaps from tomato, but it is even doubtful whether much of the sporadic infection occurring on tobacco may be accounted for in this way.

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U. S. GOVERNMENT PRINTING OFFICE: 1935

INDEX

Page

Actinomyces—
nitrogen fixation studies. Franklin E. Allison, Sam R. Hoover, and Herman J. Morris ... 1115-1123
scabies, infection of potatoes, relation to flea-beetle injury ... 251-257
ADAMS, ESTHER, and ROBBINS, WILLIAM J.: Effect of Dyes on Yeast Fermentation as Influenced by Hydrogen-Ion Concentration ... 1025-1031
Agricultural byproducts, utilization in the production of propionic acid by fermentation. H. G. Wood and C. H. Werkman ... 1017-1024
Agropyron smithii, botanical analysis, comparison of methods ... 815-842
Air, confined, effect on soil-water movement. W. L. Powers ... 1125-1133
ALBRECHT, H. R.; BRINK, R. A.; and JONES, F. R.: Genetics of Resistance to Bacterial Wilt in Alfalfa ... 635-642

Alfalfa—
bacterial wilt, genetics of resistance to. R. A. Brink, F. R. Jones, and H. R. Albrecht ... 635-642
blossom, color of inbred progenies, data ... 687-690
foliage, color of inbred progenies, data ... 690-693
leaflet—
length of inbred progenies, data ... 682-684
width of inbred progenies, data ... 684-686
leaves, changes after—
injury by live steam, study ... 397-399
potato leaf-hopper injury, study ... 392-397
plants, inbred progenies—
erectness ... 676-678
height ... 671-673
stem diameter ... 678-682
width ... 673-675
resistance to bacterial wilt, genetics of. R. A. Brink, F. R. Jones, and H. R. Albrecht ... 635-642
variability in, effects of inbreeding. George Stewart ... 669-694
ALLEN, H. W., and BURRELL, R. W.: Methods of Obtaining Emergence of *Tiphia* Adults from Imported Cocoons for Use against the Japanese Beetle ... 909-922
ALLEN, RUTH F.:
A Cytological Study of Heterothallism in Flax Rust ... 765-791
A Cytological Study of Heterothallism in *Puccinia sorghi* ... 1047-1068
ALLISON, FRANKLIN E., HOOVER, SAM R.; and MORRIS, HERMAN J.: Nitrogen Fixation Studies with Fungi and *Actinomyces* ... 1115-1123
Amino acids, content of soybean protein, determination ... 279-281
Ammonia, production by fermentation of tobacco, study ... 149-150, 157-158
Aminckia, nematosis of, caused by a new variety of *Anguillulina dipsaci*. G. Steiner and C. E. Scott ... 1087-1092

Anguillulina—
dipsaci—
hosts ... 1087
new variety of, cause of nematosis of *Aminckia*. G. Steiner and C. E. Scott ... 1087-1092
var. *aminckiae*, n. var., comparison with other forms, and description ... 1087-1092
phyllobia, n. sp., occurrence and habits, morphology ... 760-762
pratensis, distribution and host relationship ... 756-757
pustulicola, n. sp., distribution and morphology ... 757-760

Page

Anthesis, in *Sorghum* ... 123-133
Antiseptics, effect on tobacco fermentation ... 147-149, 151, 157-158
Aphids, agents in transmission of tobacco mosaic virus from different hosts. Ismé A. Hoggan ... 1135-1142
Apis mellifica, worker, imago of, changes in total nitrogen content during life ... 21-28
Apple—
fruits, penetration by petroleum oil ... 565-566, 568
leaves, penetration by petroleum oil ... 561-564, 566-567, 568
petroleum oils in, penetration, distribution and effects. Paul A. Young ... 559-571
plants, cork formations on, relation to fire-blight resistance ... 301-309, 310
resistance to fire blight, studies. Luther Shaw ... 283-313
shoots—
environment, effect on fire-blight resistance ... 290-296, 309-310
internal, host conditions, relation to fire-blight resistance ... 296-309, 310
moisture content, relation to fire-blight resistance, study ... 297-301
tissues, oils in, examples ... 564
Apples—
prestorage treatments, effect on scald and breakdown, experiments ... 57-69
soft scald and soggy break-down of. Charles Brooks and C. P. Harley ... 55-69
Artichoke byproduct, fermentation for production of propionic acid, experiments ... 1020-1022
Aspen, Minnesota, heart rot in, percentage, statistical analyses for finding a simple estimating method. R. M. Brown ... 929-942
Aspergillus, nitrogen fixation, study ... 1115-1119
Auto-irrigation, efficacy, comparison with surface irrigation ... 120
Auto-irrigator apparatus, some improvements in. L. A. Richards and H. L. Blood ... 115-121
Avocado—
inoculation with—
Botryosphaeria ribes, results ... 469, 471-472
Sphaceloma perseae, experiments ... 864-865, 866-868
scab, caused by *Sphaceloma perseae*. Anna E. Jenkins ... 859-869

Bacterial—
blight of carrot. James B. Kendrick ... 493-510
wilt, resistance in alfalfa, genetics of. R. A. Brink, F. R. Jones, and H. R. Albrecht ... 635-642
Bacterium prunі—
cause of disease of English Morello cherry. John C. Dunegan ... 745-754
pathogenicity and identification ... 746-751
BAKER, FRANCIS E., and FLEMING, WALTER E.:
Testing Contact Insecticides on the Japanese Beetle, and Results with Some Sodium and Potassium Soaps ... 29-38
The Effectiveness of Stomach-Poison Insecticides on the Japanese Beetle ... 39-44
Bark beetles, in felled Norway pine timber, relationships of blue-staining fungi to. J. G. Leach, L. W. Orr, and Clyde Christensen ... 315-341
Barley—
feeding to sows, effects on reproduction, study ... 943-952
vitamin-A deficiency, studies ... 943-952
Bean, Great Northern field, resistance to common bean mosaic. W. H. Pierce ... 183-188

BEAUDETTE, F. R.; HUDSON, C. B.; and SAXE, A. HOWARD: An Outbreak of Fowl Plague in New Jersey in 1929 — Page 83-92

Beaufort scale of wind force, adaptation for use on forested areas of northern Rocky Mountains. George M. Jemison — 77-82

BETHKE, R. M.; RECORD, P. R.; and WILDER, O. H. M.—
Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Growth Studies with Chicks — 715-722
Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Nitrogen-Balance Studies with Rats — 723-730

BLANK, L. M., and WALKER, J. C.: Fusarium-Resistant Danish Ballhead Cabbage — 983-989

Blister rust—
damage to white pine at Garibaldi, British Columbia — 239-249
white pine, spread in Pacific Northwest, relation to development of *Ribes*, studies — 93-114

BLOOD, H. L., and RICHARDS, L. A.: Some Improvements in Auto-Irrigator Apparatus — 115-121

Botryosphaeria ribis, host range—
and history — 467-468
inoculations showing. Clayton O. Smith. 467-476

Boutelova gracilis, botanical analysis, comparison of methods — 815-842

BRINK, R. A.; JONES, F. R.; and ALBRECHT, H. R.: Genetics of Resistance to Bacterial Wilt in Alfalfa — 635-642

British Columbia, Garibaldi, *Pinus monticola*, damage done by *Cronartium ribicola*. H. G. Lachmund — 239-249

BROOKS, CHARLES, and HARLEY, C. P.: Soft Scald and Soggy Breakdown of Apples — 55-69

BROWN, R. M.: Statistical Analyses for Finding a Simple Method for Estimating the Percentage Heart Rot in Minnesota Aspen — 929-942

BRUNSON, ARTHUR M., and LATSHAW, W. L.: Effect of Failure of Pollination on Composition of Corn Plant — 45-53

Bulls, dairy, gonadial tissue, transplanting results. Fred W. Miller, R. R. Graves, and G. T. Creech — 259-278

BURRELL, R. W., and ALLEN, H. W.: Methods of Obtaining Emergence of *Tiphia* Adults from Imported Cocoons for Use against the Japanese Beetle — 909-922

Butterfat percentage—
dam's and daughter's, relation between — 444-447, 462
grandparents' and granddaughters, relation between — 447-455
Jersey cattle, influence of inheritance and environment on. John W. Gowen — 433-465

Cabbage—
anatomy, normal and pathological — 613-621
Danish Ballhead, fusarium-resistant. J. C. Walker and L. M. Blank — 983-989
infection by *Plasmodiophora brassicae*, study — 608-613, 616-623
varieties, resistance to yellows, comparison — 986
Cajanus indicus, natural crossing in — 923-927
Camphor—
foliage, spraying, quantity of oil retained, study — 4-6, 13-14
scale, mortality from spraying, determination — 6-8, 11, 14-17
Carbon dioxide, formation by fermentation of tobacco — 150
Carrot—
bacterial blight—
causal organism, tests and description — 499-505
damage to seed crop — 497-499
of. James B. Kendrick — 493-510
symptoms — 493-497
seed, source of bacterial blight infection — 507-508, 509
Carrots, inoculation with bacterial blight pathogene — 505-506

Catalase—
activity, relation to age and viability of sclerotia of the cotton root-rot fungus. C. J. King, E. D. Eaton, and Claude Hope — Page 897-902
determination in sclerotia of cotton root-rot fungus, method — 898-899
Cattle—
dairy—
gonadial tissue, grafting, experiments — 259-278
heredity and environment, relative influence on milk yield and butterfat percentage — 458-461
Jersey—
assortive mating, measures for milk yield and butterfat percentage — 455-458
milk production and butterfat percentage, influence of inheritance and environment on. John W. Gowen — 433-465
Ceratostomella ips, association with bark beetles in Norway pine — 328-331
Cherry—
English Morello, disease of, caused by *Bacterium pruni*. John C. Dunegan — 745-754
leaf-spot fungus, injury, comparison with effects of *Bacterium pruni* — 746-747
Chickens, inoculation with fowl plague virus, experiments — 84-85, 86-90
Chicks—
growth studies, use to determine effect of method of manufacture on nutritive value of fish meals. P. R. Record, R. M. Bethke, and O. H. M. Wilder — 715-722
leg paralysis, experiments with various rations — 717-721
Cholodny method, use in study of soil microflora — 1099-1103, 1109-1113
CHRISTENSEN, CLYDE; LEACH, J. G.; and ORR, L. W.: The Interrelationships of Bark Beetles and Blue-Staining Fungi in Felled Norway Pine Timber — 315-341
Chromosome number and meiotic behavior in *Gossypium*. J. M. Webber — 223-237
Cinnamomum camphora, spraying in study of efficiency of oil emulsions — 1-18
Citrus—
inoculation with—
Botryosphaeria ribis, experiments — 469, 470, 471
Sphaceloma perseae, experiments — 865-866, 867
scale insects, chemical composition of, relation to their resistance to hydrocyanic acid fumigation. A. R. C. Haas — 477-492
Clover—
injury by potato leaf hopper, symptoms, artificial production — 385-392
leaflets—
excised, dry weight and respiration of, effect of mildew and rust infection on. Cecil E. Yarwood — 549-558
mildewed and rusted—
absorption of sucrose — 557-558
dry weight and respiration — 552-554
respiration, effect of sulphur dusting — 556-557
leaves, changes after—
injury by live steam, study — 397-399
potato leaf-hopper injury, study — 392-397
red, infusions, forms of nitrogen in. Walter S. Eisenmenger — 375-378
susceptibility to mildew and rust infection, experiments — 549-558
Corn—
host of *Puccinia sorghi*, studies — 1047, 1056-1058
infusions, forms of nitrogen in. Walter S. Eisenmenger — 375-378
inoculation with *Pythium arrhenomanes*, experiments — 204-210
meal, fermentation for production of propionic acid, experiments — 1020-1022
pollination, failure, effect on composition of plants. Arthur M. Brunson and W. L. Latshaw — 45-53
responses to fertility levels and seasons, studies — 991-999
root rot, variability of *Pythium arrhenomanes* in relation to. R. D. Rands and Ernest Dopp — 189-221
seedling disease, caused by *Gibberella moniliformis*, histological studies. R. K. Voorhees — 1009-1018

Corn—Continued. Page
seedlings, inoculation with *Gibberella moniliformis*, experiments 1010-1011
varieties, differential response to fertility levels and seasons. G. H. Stringfield and Robert M. Salter 991-1000
Cotton—
chromosome number and meiotic behavior 223-236
crop losses from *Phymatotrichum* root rot. Walter N. Ezekiel and J. J. Taubenhaus 843-858
pollen, storage. George J. Harrison and H. J. Fulton 891-896
root rot—
fungus—
in the soil, study by Cholodny method. E. D. Eaton and C. J. King 1109-1113
morphology and life history of, in Texas. David C. Neal, R. E. Wester, and K. C. Gunn 539-548
new sclerotia, formation 536
ozonium stage, morphological studies. 539-542
persistent strands of, in Texas. Homer C. McNamara, R. E. Wester, and K. C. Gunn 531-538
sclerotia of, age and viability, relation to catalase activity. C. J King, E. D. Eaton, and Claude Hope 897-902
sclerotial stage, morphological studies. 542-543
sclerotial stage, overwintering tests.. 546-547
hyphae—
growth from strands 536-537
overwintering stage 543-546
manurial control, microbiological activities affected. C. J. King, Claude Hope, and E. D. Eaton 1093-1107
sclerotia, longevity, relation to soil moisture. C. J. King and E. D. Eaton 793-796
yields, relation to prevalence of root rot, experiments 846-851
See also *Gossypium*.
Cows, dairy, gonadial tissue, transplanting, results. Fred W. Miller, R. R. Graves, and G. T. Creech 259-278
CRANE, H. L., and HARDY, MAX B.: Interrelation between Cultural Treatment of Pecan Trees, the Size and Degree of Filling of the Nuts, and the Composition of Kernels 643-661
CREECH, G. T.; MILLER, FRED W.; and GRAVES, R. R.: Results of Transplanting Gonadial Tissue in Dairy Cows and Bulls 259-278
CRESSMAN, A. W., and DAWSEY, LYNN H.: Oil Retention, Oil Emulsifier Ratio, and Oil-Water Ratio as Affecting the Insecticidal Efficiency of Emulsions 1-19
Cronartium ribicola—
damage to *Pinus monticola* at Garibaldi, British Columbia. H. G. Lachmund 239-249
spread in Pacific Northwest, effect of seasonal development of *Ribes* on. H. G. Lachmund 93-114
Crossing, natural, in pigeonpeas. C. P. Wilsie and Makoto Takahashi 923-927
Crucifers, wound infection and tissue invasion by *Plasmodiophora brassicae* 607-623
CSONKA, FRANK A., and JONES, D. BREESE: The Cystine, Tryptophane and Tyrosine Content of the Soybean 279-282
Cucumber mosaic virus, spread by aphids, studies 1137-1140
Currants, development, relation to spread of *Cronartium ribicola* in pine areas, studies 93-114
Cystine content of soybean. Frank A. Csonka and D. Breese Jones 279-282

Dairy cattle. *See* Cattle, dairy.
Dam, dairy, influence on milk production and butterfat percentage 440-444, 462
DAWSEY, LYNN H., and CRESSMAN, A. W.: Oil Retention, Oil-Emulsifier Ratio and Oil-Water Ratio as Affecting the Insecticidal Efficiency of Emulsions 1-19

Derris— Page
sprays, efficacy in control of gladiolus thrips, experiments 367-370, 371
use in control of gladiolus thrips, in combination with other poisons and molasses, studies. Henry H. Richardson 359-373
Diameter distributions for old-field loblolly pine stands in Maryland. G. Luther Schnur 731-743
Diets, vitamin-A-deficient, effects on reproduction of sows. E. H. Hughes 943-953
DOPP, ERNEST, and RANDS, R. D.: Variability in *Pythium arrhenomanes* in Relation to Root Rot of Sugarcane and Corn 189-221
DRECHSLER, CHARLES: *Pythium sclerotieichum*, N. Sp. Causing Mottle Necrosis of Sweetpotatoes 881-890
DUNEGAN, JOHN C.: A Disease of the English Morello Cherry Caused by *Bacterium pruni* 745-754
Dyes, effect on yeast fermentation as influenced by hydrogen-ion concentration. Esther Adams and William J. Robbins 1025-1031

EATON, E. D., and KING, C. J.—
A Study of the Cotton Root-Rot Fungus (*Phymatotrichum omnivorum*) in the Soil by the Cholodny Method 1109-1113
Influence of Soil Moisture on Longevity of Cotton Root-Rot Sclerotia 793-798
and HOPE, CLAUDE—
Catalase Activity in Relation to Age and Viability of Sclerotia of the Cotton Root-Rot Fungus 897-902
Some Microbiological Activities Affected in Manurial Control of Cotton Root Rot 1093-1107
Egg weight, relation to sexual maturity in domestic fowl. E. M. Funk 1033-1039
EISENMENGER, WALTER S.: The Forms of Nitrogen in Infusions of Corn, Timothy, Red Clover, Tobacco, and Redtop 375-378
Empoasca fabae injury, hypothesis of overaccumulation of carbohydrates 382-385, 402, 404
Emulsions—
insecticidal efficiency, effects of oil retention, oil-emulsifier ratio, and oil-water ratio. A. W. Cressman and Lynn H. Dawsey 1-19
oil concentration, effect upon insecticidal efficiency 9-12, 15-17
Environment, effect on milk yield of dairy cattle, comparison with heredity 458-461
Epitrix cucumeris—
larvae and eggs, culture 252-255
relation to common scab infection of potatoes 251-257
Erysiphe polygoni, cause of red clover mildew 549
Erwinia amylovora, resistance of rosaceous plants to, study 283-312
EZEKIEL, WALTER N., and TAUBENHAUS, J. J.: Cotton Crop Losses from *Phymatotrichum* Root Rot 843-858

FELLOWS, HURLEY, and FICKE, C. H.: Effects on Wheat Plants of *Ophiobolus graminis* at Different Levels in the Soil 871-880
Fermentation—
agricultural byproducts for production of propionic acid. H. G. Wood and C. H. Werkman 1017-1024
of tobacco, studies. James Johnson 137-160
of yeast, effect of dyes on, as influenced by hydrogen-ion concentration. Esther Adams and William J. Robbins 1025-1031
Ferrous iron, occurrence in phosphate rock. H. L. Marshall 71-76
Fertilization in *Sorghum* 133-136
Fertilizers—
effects on pecan nuts, experiments 650-660
response of corn varieties to, study 992-999
FICKE, C. H., and FELLOWS, HURLEY: Effects on Wheat Plants of *Ophiobolus graminis* at Different Levels in the Soil 871-880
Fire blight, resistance of apple and other rosaceous plants to, studies. Luther Shaw 283-313

Page

Fishmeals, nutritive value as determined by—
growth studies with chicks, relation to manufacturing method. P. R. Record, R. M. Bethke, and O. H. M. Wilder.... 715–722
nitrogen-balance studies with rats, relation to manufacturing method. O. H. M. Wilder, R. M. Bethke, and P. R. Record.... 723–730
Flax rust—
heterothallism, cytological study. Ruth F. Allen.... 765–791
sexual reproduction, comparison with other fungi.... 787–788
FLEMING, WALTER E., and BAKER, FRANCIS E.—
Testing Contact Insecticides on the Japanese Beetle, and Results with Some Sodium and Potassium Soaps.... 29–38
The Effectiveness of Stomach-Poison Insecticides on the Japanese Beetle.... 39–44
Fluorine feeding, effect on the storage of vitamin C in the rat and guinea pig. Hazel M. Hauck.... 1041–1046
Forage legumes, injury by potato leaf hopper, nature. Howard W. Johnson.... 379–406
Forest—
areas, northern Rocky Mountains, use of revised Beaufort scale of wind force. George M. Jemison.... 77–82
trees, isolated, distribution of soil moisture under. Herbert A. Lunt.... 695–703
Fowl—
domestic, egg weight, relation of sexual maturity to. E. M. Funk.... 1033–1039
plague, outbreak in New Jersey in 1929. F. R. Beaudette, C. B. Hudson, and A. Howard Saxe.... 83–92
FULTON, H. J., and HARRISON, GEORGE J.: Storage of Cotton Pollen.... 891–896
Fumigation—
by hydrocyanic acid, of citrus scale insects, relation between their chemical composition and resistance. A. R. C. Haas.... 477–492
citrus trees, resistance of scale insects to, investigations.... 477–491
Fungi—
blue-staining, in felled Norway pine timber, relationships of bark beetles to. J. G. Leach, L. W. Orr, and Clyde Christensen.... 315–341
nitrogen fixation studies. Franklin E. Allison, Sam R. Hoover, and Herman J. Morris.... 1115–1123
FUNK, E. M.: The Relation of Sexual Maturity and Egg Weight in the Domestic Fowl.... 1033–1039
Fusarium, resistance of Danish Ballhead cabbage to. J. C. Walker and L. M. Blank.... 983–989

Galls, fruit, of *Amsinckia intermedia*, caused by new variety of *Anguillulina dipsaci*, studies.... 1087–1092
Genetics of resistance to bacterial wilt in alfalfa. R. A. Brink, F. R. Jones, and H. R. Albrecht.... 635–642
Gibberella moniliformis, cause of seedling disease of corn, histological studies. R. K. Voorhees.... 1009–1015
Gladiolus thrips, control by derris, nicotine, paris green, and other poisons in combination with molasses, studies. Henry H. Richardson.... 359–373
Glucose, fermentation for production of propionic acid, experiments.... 1018, 1019
Gluten meal, fermentation for production of propionic acid, experiments.... 1019–1021, 1023
Gossypium—
aneuploidy, discussion.... 235
chromosome number and meiotic behavior. J. M. Webber.... 223–237
See also Cotton.
GOWEN, JOHN W.: The Influence of Inheritance and Environment on the Milk Production and Butterfat Percentage of Jersey Cattle.... 433–465

Page

Grain, production in corn plants, effect of pollination on, studies.... 45–53
Graphium spp., infestation of Norway pine.... 328
Grasses, mixed prairie, botanical analysis, comparison of methods.... 815–842
Grassland—
investigations in western North Dakota. vegetation, botanical analysis, comparison of methods.... 815–842
GRAVES, R. R.; MILLER, FRED W.; and CREECH, G. T.: Results of Transplanting Gonadial Tissue in Dairy Cows and Bulls.... 259–278
Great Plains, prairie grasses, botanical analysis, comparison of methods.... 815–842
Guinea pig, vitamin C storage in, effect of fluorine feeding on. Hazel M. Hauck.... 1041–1046
GUNN, K. C.; WESTER, R. E., and—
MCNAMARA, HOMER C.: Persistent Strands of the Cotton Root-Rot Fungus in Texas.... 531–538
NEAL, DAVID C. Morphology and Life History of the Cotton Root-Rot Fungus in Texas.... 539–548

HAAS, A. R. C.: Relation between the Chemical Composition of Citrus Scale Insects and Their Resistance to Hydrocyanic Acid Fumigation.... 477–492
HANSON, HERBERT C.: A Comparison of Methods of Botanical Analysis of the Native Prairie in Western North Dakota. 815–842
HARDY, MAX B., and CRANE, H. L.: Interrelation between Cultural Treatment of Pecan Trees, the Size and Degree of Filling of the Nuts, and the Composition of Kernels.... 643–661
HARLEY, C. P., and BROOKS, CHARLES: Soft Scald and Soggy Break-Down of Apples.... 55–69
HARRISON, GEORGE J., and FULTON, H. J.: Storage of Cotton Pollen.... 891–896
HAUCK, HAZEL M.: The Effect of Fluorine Feeding on the Storage of Vitamin C in the Rat and Guinea Pig.... 1041–1046
HAYDAK, MYKOLA H.: Changes in Total Nitrogen Content during the Life of the Imago of the Worker Honeybee.... 21–28
Heart rot, in Minnesota aspen, percentage of, statistical analyses for finding a simple method for estimating. R. M. Brown.... 929–942
Heat, effect on tobacco fermentation.... 147–149, 157, 158
Hellebore sprays, efficacy in control of gladiolus thrips, experiments.... 370, 371
Heredity, in dairy cattle, effect on milk yield, comparison with environment.... 458–461
Heterothallism in—
flax rust, cytological study. Ruth F. Allen.... 765–791
Puccinia sorghi, cytological study. Ruth F. Allen.... 1047–1068
Hicoria pecan. *See* Pecan.
HOGGAN, ISMÉ A.: Transmissibility by Aphids of the Tobacco Mosaic Virus from Different Hosts.... 1135–1142
Honeybee worker, imago of, changes in total nitrogen content during life. Mykola H. Haydak.... 21–28
HOOVER, SAM R.; ALLISON, FRANKLIN E.; and MORRIS, HERMAN J.: Nitrogen Fixation Studies with Fungi and *Actinomyces*.... 1115–1123
HOPE, CLAUDE; KING, C. J.; and EATON, E. D.—
Catalase Activity in Relation to Age and Viability of Sclerotia of the Cotton Root-Rot Fungus.... 897–902
Some Microbiological Activities Affected in Manurial Control of Cotton Root Rot.... 1093–1107
HORSFALL, J. G.; KERTESZ, Z. I.; and ROUSE, A. H.: Aberration in the Chemical Composition of Peas from Plants Affected with Root Rot.... 799–814

HUBBARD, V. C., and STANTON, T. R.: Influence of Smut Infection on Plant Vigor and Other Characters in Smut-Resistant Oat Varieties ... Page 903–908

HUDSON, C. B.: BEAUDETTE, F. R., and SAXE, A. HOWARD: An Outbreak of Fowl Plague in New Jersey in 1929 ... 83–92

HUGHES, E. H.: Some Effects of Vitamin-A-Deficient Diets on Reproduction of Sows ... 943–953

Humidity—
atmospheric, effect on fire-blight resistance in apple shoots ... 294–296, 309–310
effect on blooming of sorghum flowers, study ... 129–130, 131–132

HURD-KARRER, ANNIE M: Selenium Injury to Wheat Plants and Its Inhibition by Sulphur ... 343–357

Hydrocyanic acid fumigation of citrus scale insects, relation between their chemical composition and resistance. A. R. C. Haas ... 477–492

Hydrogen-ion concentration, influence upon effect of dyes on yeast fermentation. Esther Adams and William J. Robbins ... 1025–1031

Hydrol, fermentation for production of propionic acid, experiments ... 1019–1022

Inbreeding, alfalfa, effects on viability. George Stewart ... 669–694

Inheritance of—
awn development in sorghums. J. B. Sieglinger, A. F. Swanson, and J. H. Martin ... 663–668
characters in *Triticum vulgare* crosses, studies ... 573–603
color in Duroc-Jersey swine, studies ... 625–633
quality in Trumbull and Michikof varieties of winter wheat. W. W. Worzella ... 705–714

Insecticidal efficiency of emulsions, effects of oil retention, oil-emulsifier ratio, and oil-water ratio. A. W. Cressman and Lynn H. Dawsey ... 1–19

Insecticides—
contact, testing on Japanese beetle, and results with some sodium and potassium soaps. Walter E. Fleming and Francis E. Baker ... 29–38
effectiveness against gladiolus thrips, tests ... 359–372
stomach-poison, effectiveness on Japanese beetle. Walter E. Fleming and Francis E. Baker ... 39–44

Ipomoea batatas, mottle necrosis of, caused by *Pythium sclerotiichum* n. sp ... 881–890

Ips beetles, relation to blue-staining fungi in Norway pine, experiments ... 317–339

Iron, ferrous—
in phosphate rock, determination, sources of error ... 71–72
occurrence in phosphate rock. H. L. Marshall ... 71–76

Irrigation—
surface, efficacy, comparison with auto-irrigation ... 120
water, movement in soil, effect of air on ... 1125–1133

Japanese beetle—
attack on plants susceptible because of sugar content and odor of clarified extracts. F. W. Metzger, P. A. van der Meulen, and C. W. Mell ... 1001–1008
contact insecticides on, testing and results with sodium and potassium soaps. Walter E. Fleming and Francis E. Baker ... 29–38
control by use of *Tiphia* adults emerged from imported cocoons, methods. H. W. Allen and R. W. Burrell ... 909–922
effectiveness of stomach-poison insecticides on. Walter E. Fleming and Francis E. Baker ... 39–44
susceptibility to insecticides, variation during season ... 32–33

JEMISON, GEORGE M.: Beaufort Scale of Wind Force as Adapted for Use on Forested Areas of the Northern Rocky Mountains ... Page 77–82

JENKINS, ANNA E.: *Sphaceloma perseae*, the Cause of Avocado Scab ... 859–869

JOHNSON, D. W., and PALMER, L. S.: The Nutritive Properties of Protein, Vitamins B and G, and the Germ in Rye ... 169–181

JOHNSON, HOWARD W.: Nature of Injury to Forage Legumes by the Potato Leaf Hopper ... 379–406

JOHNSON, JAMES: Studies on the Fermentation of Tobacco ... 137–160

JOHNSTON, C. O., and MILLER, E. C.: Relation of Leaf-Rust Infection to Yield, Growth, and Water Economy of Two Varieties of Wheat ... 955–981

JONES, D. BREESE, and CSONKA, FRANK A.: The Cystine, Tryptophane, and Tyrosine Content of the Soybean ... 279–282

JONES, F. R., BRINK, R. A., and ALBRECHT, H. R.: Genetics of Resistance to Bacterial Wilt in Alfalfa ... 635–642

Juglandaceae, hosts of *Botryosphaeria ribis*, inoculation experiments ... 468–469, 470, 471

KENDRICK, JAMES B.: Bacterial Blight of Carrot ... 493–510

KERTESZ, Z. I., HORSFALL, J. G., and ROUSE, A. H.: Aberrations in the Chemical Composition of Peas from Plants Affected with Root Rot ... 799–814

KING, C. J., and EATON, E. D.—
A Study of the Cotton Root-Rot Fungus (*Phymatotrichum omnivorum*) in the Soil by the Cholodny Method ... 1109–1113
Influence of Soil Moisture on Longevity of Cotton Root-Rot Sclerotia ... 793–798
and HOPE, CLAUDE—
Catalase Activity in Relation to Age and Viability of Sclerotia of the Cotton Root-Rot Fungus ... 897–902
Some Microbiological Activities Affected in Manurial Control of Cotton Root Rot ... 1093–1107

LACHMUND, H. G.—
Damage to *Pinus monticola* by *Cronartium ribicola* at Garibaldi, British Columbia ... 239–249
Seasonal Development of *Ribes* in Relation to Spread of *Cronartium ribicola* in the Pacific Northwest ... 93–114

LARSON, RUSSELL H.: Wound Infection and Tissue Invasion by *Plasmodiophora brassicae* ... 607–624

LATSHAW, W. L., and BRUNSON, ARTHUR M.: Effect of Failure of Pollination on Composition of Corn Plants ... 45–53

LEACH, J. G.; ORR, L. W.; and CHRISTENSEN, CLYDE: The Interrelationships of Bark Beetles and Blue-Staining Fungi in Felled Norway Pine Timber ... 315–341

Lead arsenate—
concentrations of, effectiveness against Japanese beetle ... 41
insecticidal action on Japanese beetle, effect of stickers on ... 41–42

LECLERG, E. L.: Parasitism of *Rhizoctonia solani* on Sugar Beet ... 407–431

Legumes, forage, injury by potato leaf hopper, nature. Howard W. Johnson ... 379–406

Light—
effect on pecan root growth ... 513
influence on blooming of sorghum flowers, study ... 129–131

Liver meal, proteins, biological values, study ... 170–173

LUNT, HERBERT A.: Distribution of Soil Moisture under Isolated Forest Trees ... 695–703

Manure, application to soil for corn, response, study ... 992–1000
Manures, organic, efficacy in control of root-rot disease, experiments ... 1093–1105

Page

Manurial control of cotton root rot, microbiological activities affected. C. J. King, Claude Hope, and E. D. Eaton 1093–1107
MARSHALL, H. L.: The Occurrence of Ferrous Iron in Phosphate Rock 71–76
MARTIN, J. H.; SIEGLINGER, J. B.; and SWANSON, A. F.: Inheritance of Awn Development in Sorghums 663–668
Maryland, loblolly pine stands, old-field, diameter distributions for. Luther G. Schnur 731–743
Mating, assortive, of Jersey cattle, measures for milk yield and butterfat percentage 455–458
MCNAMARA, HOMER C.; WESTER, R. E.; and GUNN, K. C.: Persistent Strands of the Cotton Root-Rot Fungus in Texas .. 531–538
Medicago sativa. *See* Alfalfa.
Melampsora lini—
aecia, description 781–783, 786–787
life cycle, description 766–767
mycelium, description 767–769, 783–785, 786, 787–788
nonaecial infection of flax, study 772–773
spermatia, description 773–781, 785–788
spermogonia, description 769–771, 784
See also Flax rust.
MELL, C. W.; METZGER, F. W.; and VAN DER MEULEN, P. A.: The Relation of the Sugar Content and Odor of Clarified Extracts of Plants to Their Susceptibility to Attack by the Japanese Beetle. 1001–1008
METZGER, F. W.; VAN DER MEULEN, P. A.; and MELL, C. W.: The Relation of the Sugar Content and Odor of Clarified Extracts of Plants to Their Susceptibility to Attack by the Japanese Beetle 1001–1008
Mildew infection of excised clover leaflets, effect on dry weight and respiration. Cecil E. Yarwood 549–558
Milk—
fermentation for production of propionic acid 1019–1023
production, Jersey cattle, influence of inheritance and environment on. John W. Gowen 433–465
yield—
dam's and daughter's, relation between 444–447, 462
grandparents' and granddaughters', relation between 447–455
MILLER, CAREY D., and ROBBINS, RUTH C.: The Nutritive Value of Green Immature Soybeans 161–167
MILLER, E. C., and JOHNSTON, C. O.: Relation of Leaf-Rust Infection to Yield, Growth, and Water Economy of Two Varieties of Wheat 955–981
MILLER, FRED W.; GRAVES, R. R.; and CREECH, G. T.: Results of Transplanting Gonadial Tissue in Dairy Cows and Bulls 259–278
Moisture—
effect on tobacco fermentation 143–144, 157
soil, effect on fire-blight resistance in apple shoots 292–294
Molasses—
blackstrap, fermentation for production of propionic acid, experiments 1019–1023
use in control of gladiolus thrips, in combination with derris, nicotine, paris green, and other poisons, studies. Henry H. Richardson 359–373
MORRIS, HERMAN J.; ALLISON, FRANKLIN E.; and HOOVER, SAM R.: Nitrogen Fixation Studies with Fungi and *Actinomyces* 1115–1123
Mosaic—
diseases, of beans, resistence of Great Northern field variety 183–188
virus, of tobacco, transmission by aphids, to different hosts. Ismé A. Hoggan. 1137–1142
Mosquitoes, transmission of fowl plague, experiments 90–91

Page

NEAL, DAVID C.; WESTER, R. E.; and GUNN, K. C.: Morphology and Life History of the Cotton Root-Rot Fungus in Texas 539–548
Necrosis, mottle, of sweetpotatoes, caused by *Pythium scleroteichum*, n. sp. Charles Drechsler 881–890
Nemas, plant-parasitic, three new species, descriptions. Gerald Thorne 755–763
Nematosis of *Amsinckia* caused by a new variety of *Anguillulina dipsaci*. G. Steiner and C. E. Scott 1087–1092
Neotylenchus obesus, n. sp., distribution and morphology 762–763
New Jersey, fowl plague outbreak in 1929. F. R. Beaudette, C. B. Hudson, and A. Howard Saxe 83–92
Nicotine—
sprays, use in control of gladiolus thrips, experiments 361–364
use in control of gladiolus thrips, in combination with other poisons and molasses, studies. Henry H. Richardson. 359–373
Nitrogen—
balance studies with rats, indication of effect of method of manufacture on nutritive value of fishmeals. O. H. M. Wilder, R. M. Bethke, and P. R. Record 723–730
content, imago of worker honeybee, changes during life. Mykola H. Haydak 21–28
fixation, studies with fungi and *Actinomyces*. Franklin E. Allison, Sam R. Hoover, and Herman J. Morris 1115–1123
forms in infusions of corn, timothy, red clover, tobacco, and redtop. Walter S. Eisenmenger 375–378
NORDBY, JULIUS E.: White Spotting in Duroc-Jersey Swine 625–634
North Dakota, western, native prairie, botanical analysis, comparison of methods. Herbert C. Hanson 815–842

Oats, covered-smut infection, influence on plant vigor and other characteristics in smut-resistant varieties. V. C. Hubbard and T. R. Stanton 903–908
Oil—
emulsifier ratio, effect on insecticidal efficiency of emulsions. A. W. Cressman and Lynn H. Dawsey 1–19
retention, effect on insecticidal efficiency of emulsions. A. W. Cressman and Lynn H. Dawsey 1–19
sprays, effect on apples, studies 559–570
water ratio, effect on insecticidal efficiency of emulsions. A. W. Cressman and Lynn H. Dawsey 1–19
Oils, undiluted, in apple leaves, bark, and buds, symptoms 566–568, 569
Ophiobolus graminis—
at different levels in the soil, effects on wheat plants. Hurley Fellows and C. H. Ficke 871–880
depth in naturally infested field soil 872–873
infection of wheat—
field studies 879–880
greenhouse studies 873–879
ORR, L. W.; LEACH, J. G.; and CHRISTENSEN, CLYDE: The Interrelationships of Bark Beetles and Blue-Staining Fungi in Felled Norway Pine Timber 315–341
Oxalis, host of *Puccinia sorghi*, studies 1047–1060

Pacific Northwest, *Ribes*, seasonal development in relation to spread of *Cronartium ribicola*. H. G. Lachmund 93–114
Palmaceae, inoculation with *Botryosphaeria ribis*, experiments 468–469, 472, 473, 474
PALMER, L. S., and JOHNSON, D. W.: The Nutritive Properties of Protein, Vitamins B and G, and the Germ in Rye 169–181

Page

Paris green—
sprays, efficacy in control of gladiolus thrips, experiments 364-366, 371
use in control of gladiolus thrips, in combination with other poisons and molasses, studies. Henry H. Richardson 359-373
Peas—
carbohydrate content, effect of root rot 807-811, 812-813
chemical composition, effect of root rot 799-813
from plants affected with root rot, aberrations in chemical composition. Z. I. Kertesz, J. G. Horsfall, and A. H. Rouse 799-814
root rot, effect on chemical composition 799-813
Pecan—
kernels—
carbohydrate content, determination 646-647
composition, interrelation with cultural treatment of trees and size and degree of filling of nuts. H. L. Crane and Max B. Hardy 643-661
nitrogen content, determination 646
oil content, determination 646
nuts, size and degree of filling, interrelation with cultural treatment of trees and composition of kernels. H. L. Crane and Max B. Hardy 643-661
root growth—
abnormalities 527-528
and development. J. G. Woodroof and Naomi C. Woodroof 511-530
roots—
anatomy, description 519-527
growth, effect of environment on 513-519, 530
spread in soil 511-512
trees—
cultural treatment, interrelation with size and degree of filling of nuts and composition of kernels. H. L. Crane and Max B. Hardy 643-661
pruning, effects on nuts, experiments 644, 647-649, 652-655, 658
transplanted, root development 527
Peniophora gigantea, infestation of pine logs 322, 325
Percolation, rate, effect of air on 1125-1129
Petroleum oils—
penetration, distribution, and effects upon apple. Paul A. Young 559-571
toxicity to apple, discussion 568-569
Phaseolus vulgaris, susceptibility to mosaic disease, studies 183-188
Phosphate rock—
analysis for ferrous iron, methods and results 72-75
ferrous iron content. H. L. Marshall 71-76
Phymatotrichum—
omnivorum—
activities in manured and unmanured plots, experiments 1093-1105, 1109-1113
cause of cotton root-rot disease, studies 531-547
in soil, study by Cholodny method. E. D. Eaton and C. J. King 1109-1113
morphology and life history in Texas 539-547
sclerotia of, germination studies 793-798
See also Cotton root-rot fungus.
root rot, of cotton, crop losses from. Walter N. Ezekiel and J. J. Taubenhaus 843-858
Phytomonas insidiosa, cause of bacterial wilt 635
Pierce, W. H.: Resistance to Common Bean Mosaic in the Great Northern Field Bean 183-188
Pigeonpeas, natural crossing in. C. P. Wilsie and Makoto Takahashi 923-927
Pigs—
feeding with barley, experiments 946-952
gonadial tissue, grafting in dairy cattle, experiments 260, 264-267, 274-278
Pine—
loblolly, old-field stands in Maryland, diameter distributions for. G. Luther Schnur 731-743
logs, blue-stain fungi in, introduction by *Ips* spp 319-325
Norway—
felled timber, interrelationships of bark beetles and blue-staining fungi in. J. G. Leach, L. W. Orr, and Clyde Christensen 315-341

Pine—Continued. Page
Norway—Continued.
infestation by blue-staining fungus 331-335
western white, damage by *Cronartium ribicola* at Garibaldi, British Columbia 239-249
white, blister rust. *See* Blister rust.
Pinus—
monticola, damage by *Cronartium ribicola* at Garibaldi, British Columbia. H. G. Lachmund 239-249
resinosa. *See* Pine, Norway.
taeda, old-field stands in Maryland, distributions for 731-743
Pisum sativum. *See* Peas.
Plants, sugar content and odor of clarified extracts, relation to susceptibility of attack by Japanese beetle. F. W. Metzger, P. A. van der Meulen, and C. W. Meil 1001-1008
Plasmodiophora brassicae—
cause of clubroot disease 607
invasion of tissue and wound infection by. Russell H. Larsen 607-624
Poisons—
stomach, effectiveness against Japanese beetle. Walter E. Fleming and Francis E. Baker 39-44
use in control of gladiolus thrips, studies 359-372
Pollen, of cotton, storage. George J. Harrison and H. J. Fulton 891-896
Pollination—
failure in corn, effect on composition of plants. Arthur M. Brunson and W. L. Latshaw 45-53
in *Sorghum* 133-136
incomplete, of corn, effect on weights of plant and plant parts 51
Potassium oleate, effectiveness against Japanese beetle, comparison with sodium oleate 33-34
Potato—
byproducts, fermentation for production of propionic acid, experiments 1020-1022
flea beetle—
larvae as carriers of common scab fungus, investigations 255-256
relation to common scab infection of potatoes. L. A. Schaal 251-258
leaf-hopper injury—
hypothesis of overaccumulation of carbohydrates 382-385, 402, 404
hypotheses of virus and toxin 399-401
to forage legumes, nature. Howard W. Johnson 379-406
Potatoes, scab infection, common, relation of potato flea beetle to. L. A. Schaal 251-258
Powers, LeRoy: The Nature and Interaction of Genes Differentiating Habit of Growth in a Cross between Varieties of *Triticum vulgare* 573-605
Powers, W. L.: Soil-Water Movement as Affected by Confined Air 1125-1133
Prairie, native, in western North Dakota, botanical analysis, comparison of methods. Herbert C. Hanson 815-842
Propionibacterium arabinosum, use in production of propionic acid from agricultural byproducts 1017-1024
Propionic acid—
industrial production, problems 1018
production by fermentation of agricultural byproducts. H. G. Wood and C. H. Werkman 1017-1024
Protein—
nutritive properties in rye. D. W. Johnson and L. S. Palmer 169-181
rye, and liver meal, biological values 170-173
Prunus cerasus, disease of, caused by *Bacterium pruni* 745-754
Pseudaonidia duplex, mortality from spraying, determination 6-8, 11, 14-17
Pseudomonas carotae, n. sp.—
cause of bacterial wilt of carrot, tests 504
technical description 504-505
Puccinia—
sorghi—
diploidization 1052-1060
haploid infection, development 1048-1052, 1060-1061
heterothallism, cytological study. Ruth F. Allen 1047-1068

Puccinia—Continued.
triticina. *See* Wheat leaf rust. Page
Pyrethrum sprays, efficacy in control of gladiolus thrips, experiments 370–371
Pythium—
arrhenomanes—
cause of sugarcane root rot, studies 189–220
isolation method, identification, and sporulation 191–193
pathogenic relations—
with corn, experiments 204–210
with sugarcane, experiments 210–217
population studies 193–220
variability in relation to root rot of sugarcane and corn. R. D. Rands and Ernest Dopp 189–221
scleroteichum—
cultural characters 883–884
morphology 884–887
n. sp., cause of mottle necrosis of sweet-potatoes. Charles Drechsler 881–890
occurrence 881–883

QUINBY, J. R., and STEPHENS, J. C.: Anthesis, Pollination, and Fertilization in *Sorghum* 123–136

Radish, infection by *Plasmodiophora brassicae*, study 613
RANDS, R. D., and DOPP, ERNEST: Variability in *Pythium arrhenomanes* in Relation to Root Rot of Sugarcane and Corn 189–221
Rat, vitamin C storage in, effect of fluorine feeding on. Hazel M. Hauck 1041–1046
Rats, nitrogen balance studies, use to determine effect of method of manufacture on nutritive value of fishmeals. O. H. M. Wilder, R. M. Bethke, and P. R. Record 723–730
RECORD, P. R.; BETHKE, R. M.; and WILDER, O. H. M.—
Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Growth Studies with Chicks 715–722
Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Nitrogen-Balance Studies with Rats 723–730
Redtop infusions, forms of nitrogen in. Walter S. Eisenmenger 375–378
Rhizoctonia solani—
parasitism on sugar beet. E. L. LeClerg 407–431
pathogenicity studies 413–428
physiological studies 409–413
virulence on various seedling hosts, study 421–429
Ribes—
geographic distribution 97–98
leaves—
inoculation with *Cronartium ribicola*, observations 96–97, 100–111
maturity, factor in susceptibility to *Cronartium ribicola* infection, study 93–114
phenology 98–100
seasonal development, relation to spread of *Cronartium ribicola* in Pacific Northwest. H. G. Lachmund 93–114
spp.—
infection by *Cronartium ribicola* at Garibaldi, British Columbia, effect 247–249
See also Currants.
RICHARDS, L. A., and BLOOD, H. L.: Some Improvements in Auto-Irrigator Apparatus 115–121
RICHARDSON, HENRY H.: Studies of Derris, Nicotine, Paris Green, and Other Poisons in Combination with Molasses in the Control of the Gladiolus Thrips 359–373
ROBBINS, RUTH C., and MILLER, CAREY D.: The Nutritive Value of Green Immature Soybeans 161–167
ROBBINS, WILLIAM J., and ADAMS, ESTHER: Effect of Dyes on Yeast Fermentation as Influenced by Hydrogen-Ion Concentration 1025–1031
Rocky Mountains, northern, forested areas, use of revised Beaufort scale of wind force. George M. Jemison 77–82
RODENHISER, H. A.: Studies on the Possible Origin of Physiologic Forms of *Sphaerotheca sorghi* and *S. cruenta* 1069–1086

Root rot—
fungus, of cotton— Page
morphology and life history of, in Texas. David C. Neal, R. E. Wester, and K. C. Gunn 539–548
persistent strands of, in Texas. Homer C. McNamara, R. E. Wester, and K. C. Gunn 531–538
of corn, variability of *Pythium arrhenomanes* in relation to. R. D. Rands and Ernest Dopp 189–221
Rosaceae, inoculation with *Botryosphaeria ribis*, experiments 469–470, 471
Rosaceous plants, resistance to fire blight, studies. Luther Shaw 283–313
Rot, heart, in Minnesota aspen, percentage of, statistical analyses for finding, simple method for estimating. R. M. Brown 929–942
ROUSE, A. H.; KERTESZ, Z. I.; and HORSFALL, J. G.: Aberration in the Chemical Composition of Peas from Plants Affected with Root Rot 799–814
Rust—
flax. *See* Flax rust.
infection, excised clover leaflets, effect on dry weight and respiration. Cecil E. Yarwood 549–558
Rye—
amino acid, deficiencies, study 174–176
germ in, nutritive properties. D. W. Johnson and L. S. Palmer 169–181
protein, nutritive properties. D. W. Johnson and L. S. Palmer 169–181
vitamins B and G in, nutritive properties. D. W. Johnson and L. S. Palmer 169–181

Saccharomyces ceriricae, fermentation, effect of dyes on as influenced by hydrogen-ion concentration 1025–1030
SALTER, ROBERT M., and STRINGFIELD, G. H.: Differential Response of Corn Varieties to Fertility Levels and to Seasons 991–1000
SAXE, A. HOWARD; BEAUDETTE, F. R.; and HUDSON, C. B.: An Outbreak of Fowl Plague in New Jersey in 1929 83–92
Scab—
infection, common, of potatoes, relation of potato flea beetle to. L. A. Schaal 251–258
of avocados, caused by *Sphaceloma perseae*. Anna E. Jenkins 859–869
Scale insects—
control by oil emulsions, experiments 1–18
of citrus, chemical composition of, relation to their resistance to hydrocyanic acid fumigation. A. R. C. Haas 477–492
See also Camphor scale.
SCHAAL, L. A.: Relation of the Potato Flea Beetle to Common Scab Infection of Potatoes 251–258
SCHNUR, G. LUTHER: Diameter Distributions for Old-Field Loblolly Pine Stands in Maryland 731–743
Sclerotia, cotton root-rot fungus, catalase activity in relation to age and viability. C. J. King, E. D. Eaton, and Claude Hope 897–902
SCOTT, C. E., and STEINER, G.: A Nematosis of *Amsinckia* Caused by a New Variety of *Anguillulina dipsaci* 1087–1092
Scurvy, in rats and guinea pigs, relation to feeding fluorine, study 1041–1045
Selenium—
injury to wheat plants, and its inhibition by sulphur. Annie M. Hurd-Karrer 343–357
toxicity to wheat plants—
inhibition by application of sulphates and sulphur to soils 351–353
relation of selenium-sulphur to 343–351
relation of sulphate to 346–348
Sexual maturity, domestic fowl, relation to egg weight. E. M. Funk 1033–1039
SHAW, LUTHER: Studies on Resistance of Apple and Other Rosaceous Plants to Fire Blight 283–313
SIEGLINGER, J. B.; SWANSON, A. F.; and MARTIN, J. H.: Inheritance of Awn Development in Sorghums 663–668
Sires, dairy, influence on milk production and butterfat percentage 437–442, 461

SMITH, CLAYTON O.: Inoculations Showing the Wide Host Range of *Botryosphaeria ribis* — Page 467-476

Smut infection, influence on plant vigor and other characters in smut-resistant oat varieties. V. C. Hubbard and T. R. Stanton — 903-908

Soap, alkali content, effect on toxicity to Japanese beetle — 33-34

Soaps—
- potassium, of—
  - saturated fatty acids, effectiveness against Japanese beetle — 34-35
  - vegetable oils, effectiveness against Japanese beetle — 36-37
- sodium and potassium, effect on Japanese beetle. Walter E. Fleming and Francis E. Baker — 29-38

Sodium—
- fluoride, feeding to rats and guinea pigs, effects — 1041-1045
- oleate, effectiveness against Japanese beetle, comparison with potassium oleate — 33-34
- selenate, effect upon wheat plants — 344-346

Soil—
- conditions, relation to fire-blight resistance in apple plants, experiments — 290-294
- fertility levels, responses of corn to, studies — 992-999
- flora, study by Cholodny method — 1109-1113
- fungi, nitrogen fixation, studies — 1115-1121
- levels, relation to effects on wheat plants of *Ophiobolus graminis*. Hurley Fellows and C. H. Ficke — 871-880
- microflora, study by Cholodny method. 1099-1103, 1109-1113
- microorganisms, effect on cotton root rot in manured and unmanured plots, experiments — 1093-1105
- moisture—
  - control methods — 115-120
  - distribution under isolated forest trees. Herbert A. Lunt — 695-703
  - effect on pecan root growth — 513-514
  - influence on longevity of cotton root-rot sclerotia. C. J. King and E. D. Eaton — 793-796
  - source of carrot bacterial blight infection — 507-508, 509
- temperature, effect on growth of pecan roots — 516-519
- texture, effect on pecan root growth — 514
- water movement, effect of confined air on. W. L. Powers — 1125-1133

Soja max. *See* Soybeans.

Sorghum—
- anthesis, pollination and fertilization in. J. C. Stephens and J. R. Quinby — 123-136
- blooming—
  - counts per hour — 124-128
  - factors affecting — 129-133
- fertilization — 134-135
- kernel smuts. See *Sphacelotheca*.
- pollination — 133-134

Sorghums, awn development, inheritance. J. B. Sieglinger, A. F. Swanson, and J. H. Martin — 663-668

Sows, reproduction, effects of vitamin-A-deficient diets on. E. H. Hughes — 943-953

Soybean, cystine, tryptophane, and tyrosine content. Frank A. Csonka and D. Breese Jones — 279-282

Soybeans—
- green—
  - composition studies — 161-163
  - immature, nutritive value of. Ruth C. Robbins and Carey D. Miller — 161-167
  - vitamin content, determinations — 163-167
- nitrogen content, relation to amino acids — 280-281
- nutritive value, study — 279-282
- protein, amino-acid content, determination — 279-281

Sphaceloma perseae—
- cause of avocado scab. Anna E. Jenkins — 859-869
- conidial fructifications, development — 860
- cultural studies — 860-863
- technical description, varieties affected, and geographical range — 863-864

Sphacelotheca—
- *cruenta*— Page
  - cultural characters — 1080-1083
  - hybridization and segregation — 1075-1080
  - pathogenicity — 1083-1084
  - physiologic forms, possible origin, studies. H. A. Rodenhiser — 1069-1086
  - sexuality — 1071-1075
- *sorghi*—
  - cultural characters — 1080-1083
  - hybridization and segregation — 1075-1080
  - pathogenicity — 1083-1084
  - physiologic forms, possible origin, studies. H. A. Rodenhiser — 1069-1086
  - sexuality — 1071-1075

Spray residues, effectiveness in control of gladiolus thrips, tests — 359-372

Sprays, oil—
- concentration, effect upon insecticidal efficiency, experiments — 9-12, 15
- residue, determination — 4-6, 13-14

STANTON, T. R., and HUBBARD, V. C.: Influence of Smut Infection on Plant Vigor and Other Characters in Smut-Resistant Oat Varieties — 903-90

Starch, fermentation for production of propionic acid, experiment — 1018

Steam, injury to alfalfa and clover leaves, study — 387-39

STEINER, G., and SCOTT, C. E.: A Nematosis of *Amsinckia* Caused by a New Variety of *Anguillulina dipsaci* — 1087-1092

STEPHENS, J. C., and QUINBY, J. R.: Anthesis, Pollination, and Fertilization in *Sorghum* — 123-136

STEWART, GEORGE: Effects of Inbreeding on Variability in Alfalfa — 669-694

Stipa comata, botanical analysis, comparison of methods — 815-842

Stomach-poison insecticides, effectiveness on Japanese beetle. Walter E. Fleming and Francis E. Baker — 39-44

STRINGFIELD, G. H., and SALTER, ROBERT M.: Differential Response of Corn Varieties to Fertility Levels and to Seasons — 991-1000

Sucrose, absorption by mildewed and rusted clover leaflets — 557-558

Sugar beet—
- parasitism of *Rhizoctonia solani* on. E. L. LeClerg — 407-431
- roots, inoculation with *Rhizoctonia solani*, experiments — 416-420
- seedlings, damping off, relation of *Rhizoctonia solani* isolates to — 421-423

Sugarcane—
- inoculation with *Pythium arrhenomanes*, experiments — 210-217
- root rot, variability of *Pythium arrhenomanes* in relation to. R. D. Rands and Ernest Dopp — 189-221

Sulphur—
- dusting on clover leaflets, effect on respiration — 556-557
- effect on selenium injury to wheat plants. Annie M. Hurd-Karrer — 343-357

SWANSON, A. F.; SIEGLINGER, J. B.; and MARTIN, J. H.: Inheritance of Awn Development in Sorghums — 663-668

Sweetpotatoes, mottle necrosis of, caused by *Pythium sclerotichum*, n. sp. Charles Drechsler — 881-890

Swine, Duroc-Jersey—
- breeding for white spotting, experiments — 629-632, 633
- history and color relations — 625-628
- white spotting—
  - Julius E. Nordby — 625-634
  - prevalence and economic importance — 629

Taeniothrips gladioli, control by derris, nicotine, paris green, and other poisons in combination with molasses, studies — 359-373

TAKAHASHI, MAKOTO, and WILSIE, C. P.: Natural Crossing in the Pigeonpea — 923-927

TAUBENHAUS, J. J., and EZEKIEL, WALTER N.: Cotton Crop Losses from *Phymatotrichum* Root Rot — 843-858

Temperature—
- atmospheric, effect on fire-blight resistance in apple shoots — 291-292

Temperature—Continued.
effect on— Page
blooming of sorghum flowers, study. 129–130, 131
tobacco fermentation 144–147, 157
soil, effect on fire-blight resistance in apple shoots 291
Texas, cotton root-rot fungus—
in, morphology and life history. David C. Neal, R. E. Wester, and K. C. Gunn 539–548
persistent strands of. Homer C. McNamara, R. E. Wester, and K. C. Gunn 531–538
THORNE, GERALD: Some Plant-Parasitic Nemas, with Descriptions of Three New Species 755–763
Timber, Norway pine, felled, interrelationships of bark beetles and blue-staining fungi in. J. G. Leach, L. W. Orr, and Clyde Christensen 315–341
Timothy infusions, forms of nitrogen in. Walter S. Eisenmenger 375–378
Tiphia—
adults, emergence from imported cocoons, obtaining for use against the Japanese beetle, methods. H. W. Allen and R. W. Burrell 909–922
cocoons, handling for emergence of adults, methods 909–915
emergence from cocoons in cellar incubators, variations 921–922
spp., emergence from cocoons, experiments 915–921
Tobacco—
cigar-leaf, "sweating", nature of process. 137
fermentation studies. James Johnson 137–160
infusions, forms of nitrogen in. Walter S. Eisenmenger 375–378
mosaic virus—
plant hosts 1137–1140
transmissibility by aphids from different hosts. Ismé A. Hoggan 1135–1142
odors, production by fermentation 150–151
Trees, forest, isolated, distribution of soil moisture under. Herbert A. Lunt 695–703
Trifolium pratense infusions, forms of nitrogen in 375–378
Triticum vulgare—
origin, polyploid theory of, relation to factors differentiating growth habit 599–603
varieties of, growth habit of cross between, nature and interaction of genes differentiating. LeRoy Powers 573–605
See also Wheat.
Tryptophane—
content of soybean. Frank A. Csonka and D. Breese Jones 269–282
feeding to rats, experiments 174–176
Tubercularia ips, n. sp., technical description 335
Turnip, infection by *Plasmodiophora brassicae*, study 613
Tyrosine content of soybean. Frank A. Csonka and D. Breese Jones 279–282

Uromyces fallens, cause of red clover rust 549
Ustilago levis, influence on plant vigor and other characteristics in smut-resistant varieties of oats 903–908

VAN DER MEULEN, P. A.; METZGER, F. W.; and MELL, C. W.: The Relation of the Sugar Content and Odor of Clarified Extracts of Plants to Their Susceptibility to Attack by the Japanese Beetle 1001–1008
Vitamin—
A—
content of green soybeans, determination 163–164
diets deficient in, effects on reproduction of sows. E. H. Hughes 943–953
B—
content of green soybeans 164–165
nutritive properties in rye. D. W. Johnson and L. S. Palmer 169–181

Vitamin—Continued.
C— Page
content of green soybeans, determination 166–167
storage in rat and guinea pig, effect of fluorine feeding on. Hazel M. Hauck 1041–1046
G—
content of—
fishmeals, study 718, 719, 720–721
green soybeans, determination 165–166
nutritive properties in rye. D. W. Johnson and L. S. Palmer 169–181
VOORHEES, R. K.: Histological Studies of a Seedling Disease of Corn Caused by *Gibberella moniliformis* 1009–1015

WALKER, J. C., and BLANK, L. M.: Fusarium-Resistant Danish Ballhead Cabbage 983–999
Walnuts, inoculation with *Botryosphaeria ribis*, experiments 469, 470, 471
WEBBER, J. M.: Chromosome Number and Meiotic Behavior in *Gossypium* 223–237
WEREKMAN, C. H., and WOOD, H. G.: The Utilization of Agricultural Byproducts in the Production of Propionic Acid by Fermentation 1017–1024
WESTER, R. E., GUNN, K. C.; and—
MCNAMARA, HOMER C.: Persistent Strands of the Cotton Root-Rot Fungus in Texas 531–538
NEAL, DAVID C.: Morphology and Life History of the Cotton Root-Rot Fungus in Texas 539–548
Wheat—
breeding for quality improvement, experiments 708–713
environment, effect on reaction of factors differentiating growth habit 593–595
infection with leaf rust, investigations 955–980
leaf rust—
effect on—
development, experiments 970–975
water economy of plant, experiments 975–977
yield, experiments 965–970
relation to yield, growth, and water economy of two varieties of wheat. C. O. Johnston and E. C. Miller 955–981
plants—
effects of—
Ophiobolus graminis at different levels in the soil. Hurley Fellows and C. H. Ficke 871–880
sodium-selenate application to soil 344–346
selenium injury and its inhibition by sulphur. Annie M. Hurd-Karrer 343–357
spring, leaf-rust infection, experiments 958–977
two varieties, yield, growth, and water economy of, relation of leaf-rust infection to. C. O. Johnston and E. C. Miller 955–981
varieties, crosses, growth habits, interaction and nature of genes differentiating 573–603
winter—
inheritance of, quality in, studies 705–713
leaf rust infection, experiments 957–958
Trumbull and Michikof varieties, inheritance of quality. W. W. Worzella 705–714
Whey, fermentation for production of propionic acid, experiments 1019–1022
WILDER, O. H. M.; BETHKE, R. M.; and RECORD, P. R.—
Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Growth Studies with Chicks. 715–722
Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Nitrogen-Balance Studies with Rats 723–730

WILSIE, C. P., and TAKAHASHI, MAKOTO: Natural Crossing in the Pigeonpea ..... Page 923–927

Wind—
effect on blooming of sorghum flowers ... 132–133
force, Beaufort scale of, adaptation for use on forested areas of northern Rocky Mountains. George M. Jemison 77–82

WOOD, H. G., and WERKMAN, C. H.: The Utilization of Agricultural Byproducts in the Production of Propionic Acid by Fermentation 1017–1024

WOODROOF, J. G., and WOODROOF, NAOMI C.: Pecan Root Growth and Development 511–530

WOODROOF, NAOMI C., and WOODROOF, J. G.: Pecan Root Growth and Development 511–530

WORZELLA, W. W.: The Inheritance of Quality in Trumbull and Michikof Varieties of Winter Wheat ..... Page 705–714

YARWOOD, CECIL E.: Effect of Mildew and Rust Infection on Dry Weight and Respiration of Excised Clover Leaflets 549–558

Yeast fermentation—
effect of dyes on, as influenced by hydrogen-ion concentration. Esther Adams and William J. Robbins 1025–1031
for production of propionic acid, experiments 1019–1020, 1022–1023

YOUNG, PAUL A.: Penetration, Distribution, and Effect of Petroleum Oils in Apple 559–571

Zea mays. *See* Corn.